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Review Article

Food Analysis: Present, Future, and Foodomics

Alejandro Cifuentes

Laboratory of Foodomics, Institute of Food Science Research (CIAL), CSIC, Nicolas Cabrera 9, Campus de Cantoblanco, 28049 Madrid, Spain

Correspondence should be addressed to Alejandro Cifuentes, a.cifuentes@csic.es

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This paper presents a revision on the instrumental analytical techniques and methods used in food analysis together with their main applications in food science research. The present paper includes a brief historical perspective on food analysis, together with a deep revision on the current state of the art of modern analytical instruments, methodologies, and applications in food analysis with a special emphasis on the works published on this topic in the last three years (2009–2011). The article also discusses the present and future challenges in food analysis, the application of "omics" in food analysis (including epigenomics, genomics, transcriptomics, proteomics, and metabolomics), and provides an overview on the new discipline of Foodomics.

1. Food Analysis: A Brief Historical Perspective

Analysis of foods is continuously requesting the development of more robust, efficient, sensitive, and cost-effective analytical methodologies to guarantee the safety, quality, and traceability of foods in compliance with legislation and consumers' demands. The old methods used at the beginning of the 20th century based on the so-called "wet chemistry" have evolved into the current powerful instrumental techniques used in food laboratories. This improvement has led to significant enhancements in analytical accuracy, precision, detection limits, and sample throughput, thereby expanding the practical range of food applications. As mentioned by McGorrin [1] "*the growth and infrastructure of the modern global food distribution system heavily relies on food analysis (beyond simple characterization) as a tool for new product development, quality control, regulatory enforcement, and problem-solving.*" Besides, currently, there is also a huge interest in the health-related properties of foods as a result of an increasing public concern on how to improve health through the so-called functional foods, functional ingredients, and nutraceuticals. Thus, there is no doubt on the importance and current need of analytical techniques developments able to face all these demands.

Traditionally, analytical techniques have been classified according to their working principle. For example, they can be spectroscopic (e.g., mass spectrometry (MS); nuclear magnetic resonance (NMR); infrared (IR); atomic spectroscopy (AS)), biological (polymerase chain reaction (PCR); immunological techniques; biosensors), electrochemical (including also biosensors here), for separation (e.g., high-performance liquid chromatography (HPLC); gas chromatography (GC); capillary electrophoresis (CE); supercritical fluid chromatography (SFC)), for sample preparation (e.g., solid phase extraction (SPE); supercritical fluid extraction (SFE); headspace (HS); flow injection analysis (FIA); purge and trap (PAT); microwave-assisted extraction (MAE); automatic thermal desorption (ATD)), hyphenated (e.g., putting together separation and spectroscopic techniques), and so forth. Every technique provides specific information on the sample or components under study based on a specific physical-chemical interaction, and all have their own advantages and drawbacks when applied to food analysis as will be discussed below. A description of the huge number of analytical techniques commonly used in food analysis is out of the

scope of this work. An additional idea on the complexity of the number of techniques currently involved in food analysis can be obtained developing a little more one of the above subdisciplines. Considering the case of immunological techniques, they include the following ones: enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), immunodotting, radioimmunoassay (RIA), solid-phase RIA, liquid-phase RIA, immunoradiometric assay (IRMA), fluorescence: fluorescence immunoassay, enzyme-linked fluorescent immunoassay, fluorescence polarization immunoassay (FPIA), time-resolved fluorescence immunoassay (TRFI), and chemiluminescence immunoassay (CIA), up to 27 techniques can be associated to immunological techniques.

The present paper will focus on recent developments and applications of modern instrumental analytical techniques to detect compounds of significance to food science and technology, with a special emphasis on the literature published from January 2009 to January 2012. Namely, the present work will focus on modern analytical instrumentation, the development of new methods and their application in food science and technology including the new field of Foodomics [2], as well as recent works on quality control and safety, nutritional value, processing effects, storage, bioactivity, and so forth. The analysis of a large variety of food-related molecules with different chemical properties, including amino acids, peptides, proteins, phenolic compounds, carbohydrates, DNA fragments, vitamins, toxins, pesticides, additives, and chiral compounds, is also described below. Besides, a global picture on the evolution of the principal analytical techniques employed in food analysis will be provided through a comparison between the number of works published on food analysis in the first ten years of this 21st century (2001–2011) with the number of works published in the last ten years of the past 20th century (1990–2000) on the same topic.

2. Food Analysis: Current State of the Art, Methodologies, and Applications

A large number of works have directly focused on the analytical technique used in food analysis, while others have focused on the type of food, compound, or process investigated. Regarding specific analytical techniques applied to solve different problems in food analysis, one of the more active areas is the development of sample preparation techniques, in good agreement with the complex nature of foods. Sample preparation is one of the key steps for the development of any new analytical methodology; as a result, research on new sample preparation procedures is one of the most active areas in analytical chemistry. 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. This includes the development and application of molecularly imprinted polymers in food sample analysis [3], the use of monoliths in sample preparation and analysis of milk [4], the development of porous monolith microextraction techniques for determination of veterinary residues in food matrices by [5], the use of the so-called QuEChERS (quick, easy, cheap, effective, rugged, and safe) methodology for determining pesticide residues in food matrices [6], the application of immunoaffinity column clean-up techniques in food analysis [7], the development of solid-phase microextraction (SPME) techniques for quality characterization of food products [8], the application of ultrasound-assisted extraction to the determination of contaminants in food and soil samples [9], and the use of liquid phase microextraction in food analysis [10]. Besides, some papers have focused on the description of sample preparation strategies used for the analysis of aflatoxins in food and feed [11], antibacterial residues in foodstuffs [12], or the determination of pesticides in foods [13]. At present, new green sample preparation methods are being studied; among them, supercritical fluid extraction (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 [14] but also for obtaining new functional food ingredients. These extraction techniques based on pressurized fluids provide higher selectivities, shorter extraction times, and are environmentally friendly.

Following with the description based on specific analytical techniques, separation techniques continue as one of the more active areas in food analysis. Separations based on liquid chromatography (LC) are the most numerous as can be deduced from the many works published on the use of different formats of LC [15, 16], including hydrophilic interaction liquid chromatography (HILIC) [17, 18], nano-LC [10], or high-speed counter-current chromatography [19], just to name a few. Other techniques as gas chromatography (GC) still have an important role in food analysis, for instance, for the analysis of volatile fractions or fatty acids in foods [20]. Electrodriven separation techniques such as capillary electrophoresis (CE) or microchip capillary electrophoresis have found important applications in food analysis as can be deduced from the many review works devoted to this topic [21, 22], including the detection of genetically modified organisms [23, 24], nucleosides and nucleotides in foods [25], analysis of contaminants in emerging food safety issues and food traceability [26], and food-borne pathogens [27].

It is also interesting to realize how mass spectrometry (MS) has evolved in the last years in food analysis. In the 1990s, MS was mostly used as GC detector to confirm the identification of analytes. During the last decade, MS has mainly been used for direct identification and quantification of food compounds typically coupled to other separation techniques like LC and, in 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 food regulators as FDA or EFSA, especially those regarding the requested number of identification points. As a result, tandem MS has become a general tool for the 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 [28, 29]. Other MS applications include analysis of pesticides and their metabolites in food and water matrices [30, 31], analysis of food proteins and peptides [32], MS-based analytical methodologies to characterize genetically modified crops [33], MALDI-TOF MS analysis of plant proanthocyanidins [34], or multistage mass spectrometry in quality, safety, and origin of foods [35]. It is expected that new developments on ionization techniques prior to MS analysis can make even broader its application in food analysis [36].

Spectroscopic techniques are based on the principle that molecules and atoms can interact with electromagnetic radiation. Structural, physical-chemical, and/or qualitative-quantitative information of the compounds under study can thus be obtained. This information is provided by the wavelength or frequency detected in the emitted or absorbed energy spectrum. Spectroscopic techniques have found in food analysis a large use due to that they are fast, give direct measurement of the food constituents, do not use toxic reactants and solvents, can be used in process line, are not destructive and noninvasive, and some of them can detect several compounds simultaneously [37]. Nowadays, spectroscopic techniques based on infrared region are one of the most numerous in the food analysis. Thus, infrared spectroscopy is frequently used for quality control of food including analysis of honey [38] or muscle food [39]. Spectroscopic techniques based on the near infrared (NIR) spectrum have also been used to identify transgenic foods [40] or for measuring bioactive compounds in foods [41]. The use of the midinfrared region has been applied to study the secondary structure of food proteins [42] or to study intact food systems exploring their molecular structure-quality relationships [43], while raman spectroscopy has also found a number of applications in agricultural products and food analysis [44]. Another spectroscopic technique such as nuclear magnetic resonance (NMR) has been used for the rapid analysis of oil and fact content in agrifood products [45], or in the mode 31P NMR to solve different problems in food analysis [46]. Other topics currently under development are the application of chemiluminescence in food analysis [47] or the use of chemiluminescence as detection in LC [48] or CE [49]. Other spectroscopic techniques like fluorescence [50] or modern electrothermal atomic absorption spectrometry have also found interesting applications in food analysis [51].

Hyphenated techniques have found a huge number of possibilities in food analysis. This is the case of liquid chromatography online coupled to mass spectrometry (LC-MS) or tandem MS (LC-MS/MS) which have been extensively applied to ensure food safety [52, 53], particularly to analyze antimicrobials residues in food of animal origin [54], antibiotics in food samples [55], clenbuterol residues [56], food allergens [57], and so forth. Other hyphenated techniques such as gas chromatography-mass spectrometry (GC-MS) or capillary electrophoresis-mass spectrometry (CE-MS) have also found interesting applications to analyze essential oils [58] or food contaminants [59].

Biological techniques employ living organisms or some of their products such as enzymes, antibodies, and DNAs, to identify and analyze foods. Although biological-type techniques such as the classical enzymatic or microbiological analysis will not be specifically covered in this paper, their use is still a must in many microbiological and food laboratories. The main developments in this area have been brought about by the important advances in the following.

(i) DNA-based techniques and molecular methods have allowed the fast and sensitive detection of Salmonella in foods [60], the detection of fraudulent seafood species substitution [61], or the microbial composition of different foods [62, 63]. The detection of multiple genetically engineered crops [64] is still an important topic in which the effect of food processing on plant DNA degradation and PCR-based analysis of transgenic foods has also been studied [65]. These DNA-based methods also allow the authenticity determination of meat and meat products [66], the identification of animal species in food products [67], as well as the authentication of maize [68].

(ii) Biosensors are analytical devices composed of a biological recognition element (e.g., enzyme, antibody, and microbe) coupled to a chemical or physical transducer that converts the chemical signal into an electrical response. Biosensors provide a continuous or semicontinuous electronic signal proportional to an analyte or group of analytes. Biosensors have been developed for determining major and minor food components, preservatives, food colors and sweeteners, toxins, pesticides, antibiotics, and hormones [69]. They have also found use in tracking microbial contamination [70], to follow food safety [71], processing, and to certify food quality and control including the development of the so-called electronic nose [72] or electronic tongue [73] that have a large impact on flavor analysis. The advantages of these methods are the rapid response time, the high degree of specificity and sensitivity, and the possibility of being used for inline processes monitoring food manufacturing.

(iii) Other developments include the use of peptide nucleic acid (PNA)-based technologies for food analysis and food authentication [74] or the development of new immunoassay methodologies for analyzing veterinary drug residues in foods and food products [75] or to characterize plant food allergens [76].

On the other hand, many other works—instead of focusing on a given analytical technique for food analysis as above—have studied specific food constituents for whose analysis different analytical techniques and methods have been developed. One of the main topics is the case of contaminants in foods whose analysis is a must for ensuring human exposure to noxious residues though diet does not exceed acceptable levels for health. As mentioned above, the

everyday more exigent demands on food safety are bringing about a tremendous development in analytical instruments and methodologies to analyze foodborne pathogens [77] and contaminants [78–80], or the effect of food processing on pesticide residues in fruits and vegetables [81]. Good examples of the tremendous work devoted to these issues are the analysis of mycotoxins in foods [82, 83], the analysis of other toxin-like compounds as, for instance, sterigmatocystin, a toxic metabolite closely related to aflatoxins, and produced by the fungi *Aspergillus nidulans* and *A. versicolor* that has been found in different foods [84]. Another important issue is the analysis of antibiotics in foods [85], including different legal aspects related to their determination and subsequent validation of the analytical methodologies [86]. Robust analytical methods are continuously under development in order to improve the figures of merit (analysis speed, resolution, and sensitivity), allowing the fast and sensitive analysis of other food contaminants as well as possible dangerous compounds generated during food processing as acrylamide [87, 88], melatonin [89], N-nitrosamines [90], dioxins and dioxin-like PCBs [91], polycyclic aromatic hydrocarbons [92], Sudan dyes [93], food taints and off-flavours [94], thiols in wine [95], and sulphites in food and beverages [96].

Currently there is an important amount of work devoted to the study of food not only considered as source of energy, but also as a natural source of valuable ingredients than can provide additional health benefits. Following this trend new terms as functional foods, functional ingredients, or nutraceuticals are now in use in many laboratories that investigate links between the composition of food and its health benefits. This has been the case of isoflavones that has been largely analyzed in food and biological fluids based on their claimed health benefits supposedly due to their antioxidant activity [97, 98], a point that is now under controversy. Similar approach has been applied for the advanced analysis of food anthocyanins, isoflavones, and flavanols [99], flavonoids [100], phenolic compounds [101, 102], carotenoids [103, 104], and other phytochemicals in foods [105]. Many times these studies focus on the biological properties of other compounds, as fatty acids [106] or glucosinolates [107] that can have other sources different than plants. The analysis of nutraceuticals is a key issue for which advanced analytical techniques will play a key role [108] as well as for global research in nutrition and dietetics [109]. In this regard, other important topics are the determination of bioactive compounds in cranberry [110] or the determination of amino acids in grape-derived products [111]. Some compounds have also attracted much attention due to their beneficial rheological properties, this is the case of the analysis of the gelling carrageenans [112], or the determination of the degree of N acetylation of the polymers chitin and chitosan [113].

Other more specific applications in food analysis have also seen a great development as a result of the combination of several analytical advances that have been put together. A good example is the study of the geographical origin of foods via the analyses of stable isotope ratio of light elements [114] or the meta-analysis of the effects of pasteurization on milk vitamins [115]. This is also 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 gas chromatography and related techniques; (iii) new analytical techniques, such as comprehensive gas chromatography (GC); (iv) new operative strategies based on approaches developed for other fields and applied to food analysis; (v) data elaboration strategies producing a higher level of information [116]. 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, and polyphenols [117]. Another example is the investigation on food texture in which physical characteristics perceived by the senses are investigated. Research in this area has evolved tremendously in the last decade based on multidisciplinary approaches that encompass chemistry, physics, physiology, and psychology, to study fracture of food, the sounds it makes during biting and chewing, its microstructure, muscle movements during mastication, swallowing, and acceptability, and so forth [118, 119].

Several books have also been printed in this period focusing on molecular techniques for detection and characterization of foodborne pathogens [120, 121], molecular biological and immunological techniques for food chemists [122], mass spectrometry in food safety [123], food analysis instruments [124], dairy food analysis [125], grains identity preservation and traceability [126], food contaminants [127], antibiotic residues in food [128], and fortified foods with vitamins [129].

Also, several book chapters have focused on the use of FTIR for rapid authentication and detection of adulteration of food [130], time-domain NMR applied to food products [131], or biosensors for functional food safety and analysis [132]. Readers interested in this topic can also take resort of several special issues published by different journals on food analysis [133], advanced separation methods in food analysis [134], allergens in foods [135], natural bioactive compounds and nutrigenomics [136], food and beverage analysis [137] and advanced food analysis [138].

Table 1 provides 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 conclusions that can be extracted from the results of Table 1 when they are compared to the results from a similar search published by our group at the end of the 20th century summarizing the works published on food analysis in the period 1990–2000 [139]. The most important trend is the huge increase in biological

Table 1: 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.

Technique	Number of ref.
Sample preparation	
Microwave-assisted extraction	384
Headspace	2571
Solid phase extraction	3866
Supercritical fluid extraction	680
Purge and trap	151
Flow injection analysis	393
Pressurized liquid extraction	436
Microextraction	2201
Biological	
Biosensors	750
PCR	7085
Microbiological analysis	416
Recombinant DNA	220
Immunological techniques	3008
Others[a]	118
Separation	
Liquid chromatography	8927
Gas chromatography	4798
SDS/PAGE	3227
Capillary electrophoresis	1155
Supercritical fluid chromatography	51
LC × LC, LC-LC	27
GC × GC, GC-GC	210
LC-GC	38
Spectroscopic	
Mass spectrometry	5030
Fluorescence	4807
NMR	3735
Infrared	2369
X-ray	2119
Ultraviolet	1429
Atomic spectroscopy	1046
Electron spectroscopy	1026
Light scattering	891
Circular dichroism	468
Others[b]	932
Rheological	
Creep	205
Oscillatory shear	203
Rheometry	195
Viscometry	163
Stress relaxation	145
Normal stress	32
Thermal	
DSC	551
Thermogravimetry	17
The mochemical	16
Differential thermal analysis	9
Radiochemical	
Radioimmunoassay	96
Isotopic	140
Radiochemical	31
Radiometric	18
Radioisotope	8
Radiotracer	5
Radiolabelling	2
Electrochemical	
Biosensors	750
Voltammetry	532
Potentiometry	234
Amperometry	245
Polarography	47
Conductometry	43
Coulometry	32

[a]The group "others" includes radioimmunoassay and enzymatic analysis.
[b]The group "others" includes raman (402), electron spin resonance (366), dielectric spectroscopy (57), refractometry (54), polarimetry (38), chemiluminescence (15), and photoacoustic (0).

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 two, three, and four times, respectively. Other well-established techniques such as separation techniques continue to be used in a high extend, but nowadays they are not the most widely used (as in the period 1990–2000), since spectroscopic techniques have gained importance and at present are the most extensively used in food analysis.

3. Present Challenges in Food Analysis

Currently there are a good number of challenges to be solved in food analysis. The variety of toxic residues in food is continuously increasing as a consequence of industrial development, new agricultural practices, environmental pollution, and climate change. A critical issue will be how to detect untargeted compounds and determine their identity in foods, for which the development of advanced

analytical techniques is expected to play a crucial role [140]. This increasing number of food contaminants is bringing about the development of everyday more powerful, sensitive, and fast analytical methodologies able to detect emerging contaminants in food-like industrial organic pollutants, nanomaterials, pharmaceutical residues antibiotics, and coccidiostats or emerging groups of marine biotoxins [141]. In spite of these important developments, 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. Microbiologists have developed over the last decades reliable culture-based techniques for pathogens detection in foods. These methods are considered to be the "gold-standard"; however, they remain cumbersome and time consuming. The introduction of genetic-based technologies makes 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 [142, 143]. 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 [144]. 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 [78].

It is also now under discussion the way in which the toxicity of food chemical contaminants is addressed. It seems it should be optimized considering food cooking, processing and eating habits, parameters that usually are neglected [145]. In addition, there is also a need of new and alternative (in vivo) toxicity tests as well as new detection tools for testing the effects of food chemical contaminants, moving away from the mouse bioassay for food toxin analysis [146]. To achieve this goal, the use of noncarcinogenic functional cell models, of alternative animal models like zebrafish embryos, and a toxicogenomic approach, seem to be the most promising strategies for the future toxicity assessment of food chemical contaminants [147].

There is also a good amount of discussion on how to ensure food safety around the globe and the many roles of risk analysis including microbial risk assessment [148, 149]. In this regard, benefit-risk assessment in food and nutrition weighs the beneficial and adverse effects that a food (component) may have, in order to facilitate more informed management decisions regarding public health issues. Although benefit-risk assessment is now in its infancy, and its exact scope is yet to be defined, benefit-risk assessment can be a valuable approach to provide the best possible science-based answer to complicated questions with a large potential impact on public health [150]. To achieve these goals it will be necessary to develop new and more powerful tools for the performance assessment and improvement of food safety following a global approach [151]. For instance, a good topic to evaluate through benefit-risk assessment will be the use of nanomaterials in food science. 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; improved colors and flavors. Based on 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 [152]. Besides, nanomaterials are also expected to play a crucial role in the miniaturization of analytical systems [153] including newly emerging technologies able to offer platforms with greater automation and multiplexing capabilities [154] or in the development of new nanomaterial-based biosensors for food analysis applications [155]. 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 [156]. 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 [157]. Miniaturized systems and their applications are expected to keep growing in food analysis.

More suitable analytical techniques are still required for the detection of allergens in foods because minute amounts of the allergen can have critical consequences in sensitized persons. Although immunological methods are currently preferred, the determination of allergenic proteins by LC-MS has advanced in recent years, and it is now frequently used for the identification and quantitation of food allergens [57]. In spite of these advances, confirmatory alternatives are still needed to be able to face other additional problems originated, for example, by food matrix interferences or food processing, which may not influence allergenicity but do impair allergen detection.

Regarding comprehensive and multidimensional chromatography methods (e.g., LC $\times$ LC; GC $\times$ GC), an important point to consider is that nowadays these multidimensional techniques require dedicated laboratories, equipment, and highly trained personnel, what can explain the relatively low number of applications of these techniques [159–161]. In order to improve the simplicity and speed of analysis provided by these techniques, keeping the cost of analysis as low as possible, important instrumental developments

have to be achieved. They include the improvement of the connection of the two systems to overcome 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 [159].

Nowadays, food laboratories are being pushed to exchange their classical procedures for modern analytical techniques that allow them to give an adequate answer to global demands on food safety, quality, and traceability. Besides, the Montreal Protocol has had a major impact also on food laboratories that have been pushed to develop more environmental-friendly methods. An important challenge is the implementation of Green Analytical Chemistry [162] also in food analysis laboratories [163] including, for instance, 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.

4. Food Analysis in the Postgenomic Era: Omics Approaches and Foodomics

The sequencing of nearly the whole human genome at the beginning of 21st century is considered the beginning of the so-called postgenomic era. The advances observed in this period have made feasible to count on analytical instruments and methodological developments that were unthinkable few decades ago. As a result, researchers in food science and nutrition are being pushed to move from classical methodologies to more advanced strategies usually borrowing methods well established in medical, pharmacological, and/or biotechnology research. In this context, we coined and defined *Foodomics* as a *discipline that studies the food and nutrition domains through the application of advanced omics technologies to improve consumer's well-being, health, and knowledge* [2, 21, 164]. The main idea behind the use of this new term has been not only to use it as a flag of the new times for food analysis, but also to highlight that the investigation into traditional and new problems in food analysis in the postgenomic era can find exciting opportunities and new answers through the use of epigenomics, genomics, transcriptomics, proteomics, and metabolomics tools. A description on the basis of these omics approaches is given next.

Epigenomics studies the mechanisms of gene expression that can be maintained across cell divisions, and thus the life of the organism, without changing the DNA sequence. The epigenetic mechanisms are related to changes induced (e.g., by toxins or bioactive food ingredients) in gene expression via altered DNA methylation patterns, altered histone modifications, or noncoding RNAs, including small RNAs. The global analysis of gene expression as approached by transcriptomics offers impressive opportunities in Foodomics (e.g., for the identification of the effect of bioactive food constituents on homeostatic regulation and how this regulation is potentially altered in the development of certain chronic diseases). Two conceptually different analytical approaches have emerged to allow quantitative and comprehensive analysis of changes in mRNA expression levels of hundreds or thousands of genes. One approach is based on microarray technology, and the other group of techniques is based on DNA sequencing. Next, typically real-time-PCR is applied to confirm the up- or downregulation of a selected number of genes. In proteomics, the huge dynamic concentration range of proteins in biological samples causes many detection difficulties due to that many proteins are below the sensitivity threshold of the most advanced instruments. For this reason, fractionation and subsequent concentration of the proteome are often needed. Besides, the use and development of high-resolving separation techniques as well as highly accurate mass spectrometers are nowadays essential to solve the proteome complexity. Two fundamental analytical strategies can be employed: the *bottom-up* and the *top-down* approach. Both methodologies differ on the separation requirements and the type of MS instrumentation. New proteomic approaches based on array technology are also being employed. Metabolome can be defined as the full set of endogenous or exogenous low molecular weight metabolic entities of approximately <1000 Da (metabolites), and the small pathway motifs that are present in a biological system (cell, tissue, organ, organism, or species). Unlike nucleic acid or protein-based omics techniques, which intend to determine a single chemical class of compounds, metabolomics has to deal with very different compounds of very diverse chemical and physical properties. Moreover, the relative concentration of metabolites in the biological fluids can vary from millimolar level (or higher) to picomolar, making it easy to exceed the linear range of the analytical techniques employed. No single analytical methodology or platform is applicable to detect, quantify, and identify all metabolites in a biological sample. Two analytical platforms are currently used for metabolomic analyses: MS- and NMR-based systems. These techniques either stand alone, or combined with separation techniques (typically, LC-NMR, GC-MS, LC-MS, and CE-MS), can produce complementary analytical information to attain more extensive metabolome coverage. There are three basic approaches that can be used in metabolomics: target analysis, metabolic profiling, and metabolic fingerprinting. Target analysis aims the quantitative measurement of selected analytes, such as specific biomarkers or reaction products. Metabolic profiling is a nontarget strategy that focuses on the study of a group of related metabolites or a specific metabolic pathway. Meanwhile, metabolic fingerprinting does not aim to identify all metabolites, but to compare patterns of metabolites that change in response to the cellular environment.

Due to the huge amount of data usually obtained from omics studies, it has been necessary to develop strategies to convert the complex raw data obtained into useful information. Thus, bioinformatics has become also a crucial tool in Foodomics. Over the last years, the use of biological knowledge accumulated in public databases by means of bioinformatics allows to systematically analyse large data lists in an attempt to assemble a summary of the

most significant biological aspects. Also, statistical tools are usually applied, for example, for exploratory data analysis to determine correlations among samples (which can be caused by either a biological difference or a methodological bias), for discriminating the complete data list and reduce it with the most relevant ones for biomarkers discovery, and so forth

The application of advanced omics technologies as proposed by Foodomics has already found application in different topics in food science and nutrition. Some examples are given below. Proteomics and metabolomics represent powerful analytical strategies to acquire more detailed and complete information on food composition even beyond the traditional food component analysis. Following this idea, proteomic methods have already been applied to several issues related to food safety [165], as well as the optimization of processing and quality control of food of animal origin [166], to investigate the milk proteome [167, 168], the rice proteome [169], or to study allergy and intolerance to wheat products [170]. Transcriptomic, proteomic, nd metabolomic approaches 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, and so forth. For instance, in the context of food safety and transcriptomics, several DNA microarray chips have already been developed for the detection of food-borne pathogens, toxigenic microorganisms, GMO analysis, and so forth. Proteomic and metabolic changes also occur during crops growing conditions, food processing/preparation (fermentation, baking, boiling, etc.), and 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 matrices [171–173] including the analysis of GM foods. 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, and labelling issues) has already been recommended by the European Food Safety Authority [33, 174–176]. 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 [177]. There are also other topics in which Foodomics can play a crucial role including the comprehensive assessment of food safety, quality and traceability ideally as a whole [164]; the investigation on the global role and functions of gut microbiome, a topic that is expected to open an impressive field of research [178, 179]; the stress adaptation responses of food-borne pathogens to ensure food hygiene, processing and preservation [180], or 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 [181].

Currently, a main area of research in food science is the connection between food and health. Today, food is considered 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, for example, 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. Interaction of modern food science and nutrition with disciplines such as pharmacology, medicine, or biotechnology provides impressive new challenges and opportunities. As a result, advanced analytical methodologies, "omics" approaches, and bioinformatics—frequently together with in vitro, in vivo, and/or clinical assays—are applied to investigate topics in food science and nutrition that were considered unapproachable few years ago demonstrating the huge possibilities of Foodomics in this important area of research [182]. However, food scientists and nutritionists have to face a large number of challenges to adequately answer the new questions emerging from this new field of research. One of the main challenges is to improve our limited understanding of the roles of nutritional compounds at 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. The problem to solve is huge, and it includes the study of the individual variations in gene sequences, particularly in single nucleotide polymorphisms (SNPs), and their expected different answer to nutrients. Moreover, nutrients can be considered as signalling molecules that are recognized by specific cellular-sensing mechanisms [183]. However, unlike pharmaceuticals, the simultaneous presence of a variety of nutrients, with diverse chemical structures and concentrations and having numerous targets with different affinities and specificities, increases enormously the complexity of the problem [184]. Therefore, it is necessary to look at hundreds of test compounds simultaneously and observe the diverse temporal and spatial responses. Besides, several of the health benefits assigned to many dietary constituents are still under controversy as can be deduced from the large number of applications rejected by the European Food Safety Authority about health claims of new foods and ingredients [185]. More sounded scientific evidences are needed to demonstrate or not the claimed beneficial effects of these new foods and constituents. In this sense, the advent of new postgenomic strategies as Foodomics seems to be essential to understand how the bioactive compounds from diet interact at molecular and cellular level, as well as to provide better scientific evidences on their health benefits. The combination of the information from the three expression levels (gen, protein, and metabolite) can be crucial to adequately understand and scientifically sustain the health benefits from food ingredients. 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 1. Following this Foodomics strategy, results on the effect of food ingredient(s) at genomic/transcriptomic/proteomic and/or metabolomic level are obtained, making possible new investigations on food bioactivity and its effect on human health at molecular level [186].

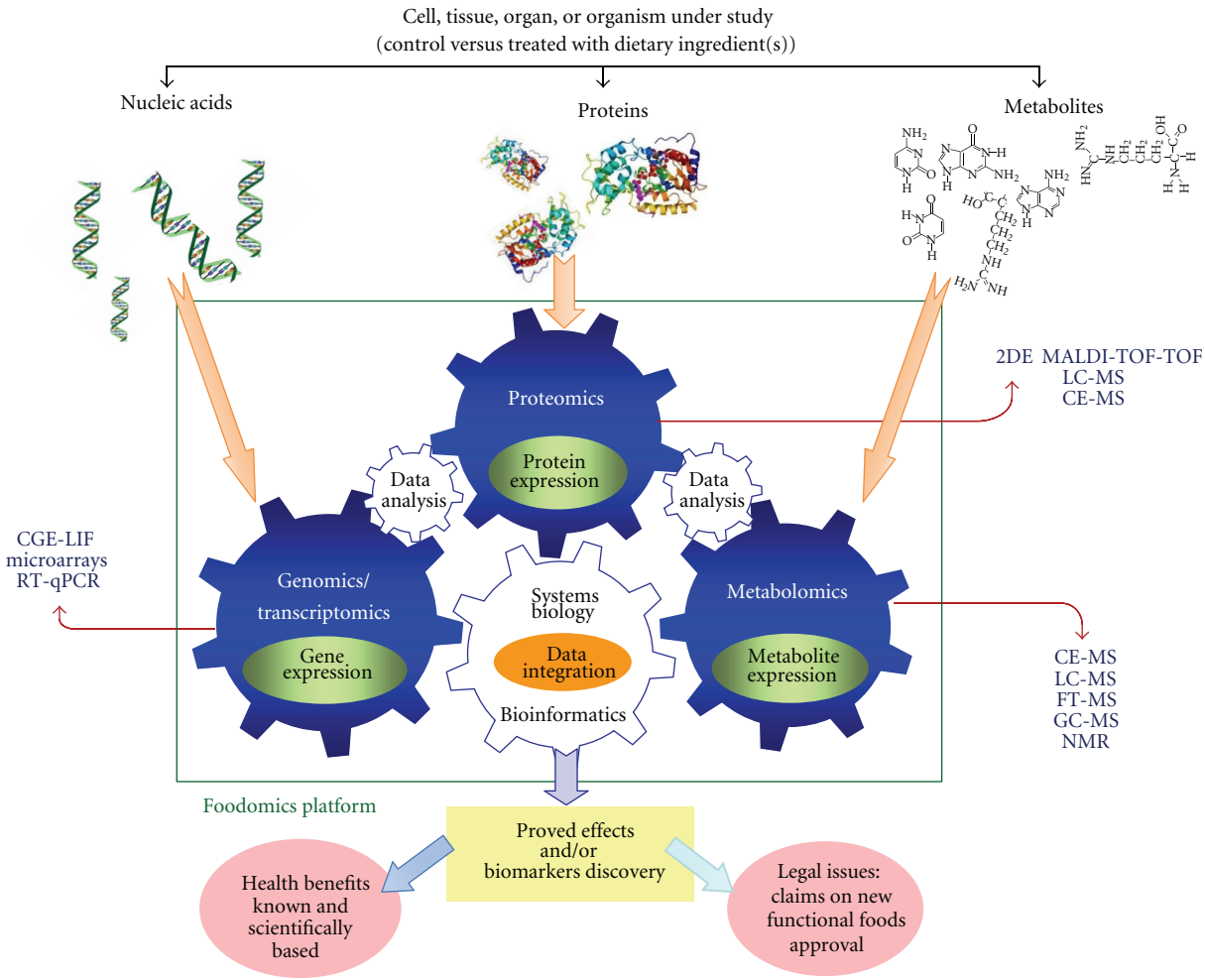

Figure 1: Scheme of an ideal Foodomics platform to investigate the health benefits from dietary constituents on a given biological system (cell, tissue, organ, or organism), including analytical methodologies used and expected outcomes. Modified from [158] with permission from Elsevier.

However, in spite of the significant outcomes expected from global Foodomics strategies, there is still a long way to go since practically there are no papers published in literature in which results from the three expression levels (transcriptomics, proteomics, and metabolomics) are simultaneously presented and merged being the number of review papers on this topic higher than the number of research papers. To our knowledge, only two papers have presented results from the three expression levels simultaneously following a global strategy [158, 187]. Figure 2 shows the results from one of these global Foodomics studies on the chemopreventive effect of dietary polyphenols against HT29 colon cancer cells proliferation [158]. Figure 2 shows the genes, proteins and metabolites that were identified (after transcriptomic, proteomic, and metabolomic analysis) to be involved in the principal biological processes altered in HT29 colon cancer cells after the treatment with rosemary polyphenols, showing the huge potential of this global strategy.

However, Foodomics still needs to overcome some important gaps and limitations in order to demonstrate all its great analytical potential. Analytical strategies used in Foodomics have to face important difficulties derived, among others, from food complexity, the huge natural variability, the large number of different nutrients and bioactive food compounds, their very different concentrations, and the numerous targets with different affinities and specificities that they might have. In this context, epigenomics, transcriptomics, proteomics, and metabolomics represents powerful analytical platforms developed for the analysis of gene expression, proteins, and metabolites. However, "omics" platforms must be integrated in order to understand the biological meaning of the results on the investigated system (e.g., cell, tissue, organ) ideally through a holistic strategy as proposed by Systems Biology. Thus, Systems Biology can be defined as an integrated approach to study biological systems, at the level of cells, organs, or organisms, by measuring and integrating genomic, proteomic, and metabolic data [188]. Systems Biology approaches might encompass molecules, cells, organs, individuals, or even ecosystems, and it is regarded as an integrative approach of all information at the different levels of genomic expression (mRNA, protein, and metabolite). Data interpretation and integration when

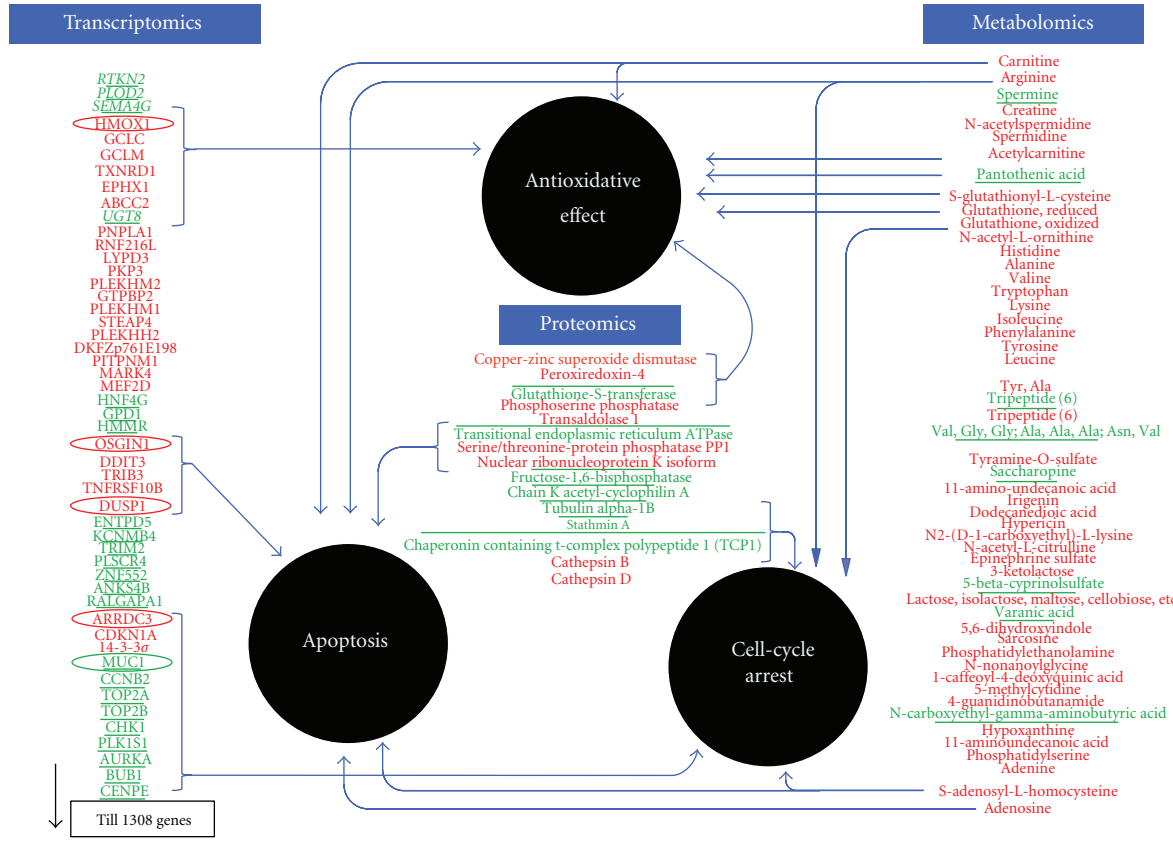

Figure 2: Foodomics identification of the genes, proteins and metabolites involved in the principal biological processes altered in HT29 colon cancer cells after their treatment with rosemary polyphenols. In red: up-regulated; in green: down-regulated. Modified from [158] with permission from Elsevier.

dealing with such complex systems are not straightforward and have been detected as one of the main bottlenecks. In the future, the combination of Foodomics and systems biology can provide crucial information on, for example, host-microbiome interactions, nutritional immunology, food microorganisms including pathogens resistance, farm animal production, or 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 [181]. Besides, it is foreseen the emerging of other innovative approaches as, for example, green Foodomics, green systems biology [189], the human-gutome, and so forth.

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