Foodomics
Advanced Mass Spectrometry in Modern Food Science and Nutrition

Edited by Alejandro Cifuentes
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FOODOMICS

Advanced Mass Spectrometry in Modern Food Science and Nutrition

Edited by

ALEJANDRO CIFUENTES
Laboratory of Foodomics (CIAL)
National Research Council (CSIC)
Madrid, Spain
To the three women in my life, Susana, Claudia and Fernanda, every day they make of this world a better place to be.

A las tres mujeres de mi vida, Susana, Claudia y Fernanda, porque cada día ellas hacen de este mundo un lugar mejor donde vivir.
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The impressive analytical developments achieved at the end of the twentieth century have made possible the sequencing of nearly the whole human genome at the beginning of the twenty-first century, opening the so-called *postgenomic era*. These advances have made feasible analytical instruments and methodological developments that were unthinkable a few decades ago. These impressive developments have traditionally found their first application in the biotechnological or biochemical field many times linked to pharmaceutical, medical, or clinical needs. The huge amount of money allocated to these fields of research is logically an additional push to be considered when selecting the area in which a new analytical method can be probed, a good way to compensate the efforts behind any innovative analytical development. As a result, biotech, pharmaceutical, and clinical related industries have usually been the first targets for analytical chemists and instrumentation companies. This has left food analysis overshadowed and connected to the use of more traditional analytical approaches. Nowadays, boundaries among the different research fields are becoming more and more diffuse giving rise to impressive possibilities in the emerging interdisciplinary areas, for example, health and food. 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. This trend has generated the emergence of new areas of research for which a new terminology is required. In this context, our group defined a few years ago *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 confidence*. 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 genomics, transcriptomics, epigenetics, proteomics, and metabolomics tools. Indeed, Foodomics is opening a new and unexpected land still wild, still unexplored, to a new generation of researchers who, using the everyday more powerful omics technologies, can find original search possibilities and innovative answers to crucial questions not only related to food science but also related to its complex links with our health.

The interest of the scientific community in modern food analysis and Foodomics, and the different trends in this hot area of research are well documented in the 20 chapters that compose this volume on “Foodomics: Advanced Mass Spectrometry in Modern Food Science and Nutrition”, the first book devoted to this new discipline in which the authors present their advanced perspective of the topic. Namely, in the first chapter the principles of Foodomics are presented, the next five chapters (chapter 2 to 6) are devoted to proteomics applications in Foodomics, including a description of modern instruments and methods for proteomics, proteomic-based techniques for food science and food allergens characterization, examination of antioxidant food supplements using advanced proteomics methods and proteomics in nutritional systems biology. The next two chapters (chapters 7 and 8) are devoted to the description of advanced MS-based methodologies to study transgenic foods development and characterization and the microbial metabolome. The following nine chapters (chapters 9 to 17) are devoted to metabolomics developments in Foodomics with special emphasis on the possibilities of MS-based metabolomics in nutrition and health research, for food safety, quality, and traceability, the investigations on future personalized nutrition, the study of the effect of the diet on acute and endurance exercise, the investigation on diet-related diseases, and the study on how Foodomics impact optimal nutrition or can provide crucial information on micronutrients (the case of folates), phenolic compounds as functional ingredients, and lipids (lipidomics). The following two chapters (chapters 18 and 19) present the main principles of Green Foodomics and the use of chemometrics in mass spectrometry and Foodomics. The last chapter of the book is devoted to the description of the possibilities of systems biology in food and nutrition research.

As editor of this book devoted to “Foodomics: Advanced Mass Spectrometry in Modern Food Science and Nutrition”, I would like to thank all the authors for their suitable contributions, Dom Desiderio for inviting me to prepare this piece of work, Michael Leventhal for his help and support, and to those in the John Wiley & Sons team who contributed their effort to the preparation of this volume.

Alejandro Cifuentes
CONTRIBUTORS

Francesco Addeo, Dipartimento di Scienza degli Alimenti, University of Naples Federico II, Naples, Italy

Juan Pablo Albar, Functional Proteomics Group, Centro Nacional de Biotecnología–CSIC, Madrid, Spain

Lluís Arola, Centre Tecnològic de Nutrició i Salut (CTNS), TECNIO, Reus, Spain; Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Universitat Rovira i Virgili, Tarragona, Spain

Anna Arola-Arnal, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Universitat Rovira i Virgili, Tarragona, Spain

Coral Barbas, Center for Metabolomics and Bioanalysis (CEMBIO), Facultad de Farmacia, Universidad CEU San Pablo, Boadilla del Monte, Madrid, Spain

Isabel Bondia-Pons, Quantitative Biology and Bioinformatics, VTT Technical Research Centre of Finland, Espoo, Finland

Antoni Caimari, Centre Tecnològic de Nutrició i Salut (CTNS), TECNIO, Reus, Spain

Mónica Carrera, Institute of Molecular Systems Biology, ETH Zürich, Zürich, Switzerland

María Castro-Puyana, Laboratory of Foodomics, Institute of Food Science Research (CIAL), National Research Council (CSIC), Madrid, Spain
Alejandro Cifuentes, Laboratory of Foodomics, Institute of Food Science Research (CIAL), National Research Council (CSIC), Madrid, Spain

Sebastiano Collino, BioAnalytical Science, Nestle Research Center, Lausanne, Switzerland

Anna Crescenti, Centre Tecnòlogic de Nutrició i Salut (CTNS), TECNIO, Reus, Spain

Josep M. del Bas, Centre Tecnològic de Nutrició i Salut (CTNS), TECNIO, Reus, Spain

Sylvia H. Duncan, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK

Susan J. Duthie, Natural Products Group, Division of Lifelong Health, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK

Søren B. Engelsen, Faculty of Science, University of Copenhagen, Copenhagen, Denmark

Marcela A. Erazo, Center for Metabolomics and Bioanalysis (CEMBIO), Facultad de Farmacia, Universidad CEU San Pablo, Boadilla del Monte, Madrid, Spain

Laurent Fay, R&D Infant Formulae, Nestlé Nutrition, Vevey, Switzerland

Pasquale Ferranti, Istituto di Scienze dell’Alimentazione, CNR, Avellino, Italy; Dipartimento di Scienza degli Alimenti, University of Naples Federico II, Naples, Italy

Federico Ferreres, Department of Food Science and Technology, CEBAS-CSIC, Murcia, Spain

Jose M. Gallardo, Marine Research Institute, CSIC, Vigo, Pontevedra, Spain

Antonia García, Center for Metabolomics and Bioanalysis (CEMBIO), Facultad de Farmacia, Universidad CEU San Pablo, Boadilla del Monte, Madrid, Spain

Virginia García-Cañas, Laboratory of Foodomics, Institute of Food Science Research (CIAL), National Research Council (CSIC), Madrid, Spain

Cristina García-Viguera, Department of Food Science and Technology, CEBAS-CSIC, Murcia, Spain

José Ignacio Gil, Service of Radiodiagnostic, Mammary Pathology Department, Hospital José María Morales Meseguer, Murcia, Spain

Angel Gil-Izquierdo, Department of Food Science and Technology, CEBAS-CSIC, Murcia, Spain

Jean-Philippe Godin, BioAnalytical Science, Nestle Research Center, Lausanne, Switzerland
Miguel Herrero, Laboratory of Foodomics, Institute of Food Science Research (CIAL), National Research Council (CSIC), Madrid, Spain

Tuulia Hyötyläinen, Quantitative Biology and Bioinformatics, VTT Technical Research Centre of Finland, Espoo, Finland

Clara Ibáñez, Laboratory of Foodomics, Institute of Food Science Research (CIAL), National Research Council (CSIC), Madrid, Spain

Elena Ibáñez, Laboratory of Foodomics, Institute of Food Science Research (CIAL), National Research Council (CSIC), Madrid, Spain

Elsa M. Janle, Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana, USA

Peter Kastenmayer, BioAnalytical Science, Nestle Research Center, Lausanne, Switzerland

Martin Kussmann, Proteomics/Metabonomics Core, Nestlé Institute of Health Sciences, Lausanne, Switzerland; Faculty of Science, Aarhus University, Aarhus, Denmark

Ashraf G. Madian, Department of Chemistry, Purdue University, West Lafayette, Indiana, USA

Gianfranco Mamone, Istituto di Scienze dell’Alimentazione, CNR, Avellino, Italy

François-Pierre Martin, BioAnalytical Science, Nestle Research Center, Lausanne, Switzerland

Sonia Medina, Department of Food Science and Technology, CEBAS-CSIC, Murcia, Spain

María del Carmen Mena, Functional Proteomics Group, Centro Nacional de Biotecnología–CSIC, Madrid, Spain

José A. Mendiola, Laboratory of Foodomics, Institute of Food Science Research (CIAL), National Research Council (CSIC), Madrid, Spain

Sofía Moco, BioAnalytical Science, Nestle Research Center, Lausanne, Switzerland

Chiara Nitride, Dipartimento di Scienza degli Alimenti, University of Naples Federico II, Naples, Italy

Matej Orešič, Systems Biology and Bioinformatics, VTT Technical Research Centre of Finland, Espoo, Finland

Ignacio Ortea, Health Research Institute of Santiago de Compostela, A Coruña, Spain
CONTRIBUTORS

Gianluca Picariello, Istituto di Scienze dell’Alimentazione, CNR, Avellino, Italy

Francesc Puiggròs, Centre Tecnològic de Nutrició i Salut (CTNS), TECNIO, Reus, Spain

Fred E. Regnier, Department of Chemistry, Purdue University, West Lafayette, Indiana, USA

Serge Rezzi, BioAnalytical Science, Nestle Research Center, Lausanne, Switzerland

Alastair Ross, BioAnalytical Science, Nestle Research Center, Lausanne, Switzerland

Francisco J. Rupérez, Center for Metabolomics and Bioanalysis (CEMBIO), Facultad de Farmacia, Universidad CEU San Pablo, Boadilla del Monte, Madrid, Spain

Wendy R. Russell, Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK

Max Scherer, BioAnalytical Science, Nestle Research Center, Lausanne, Switzerland

Thomas Skov, Faculty of Science, University of Copenhagen, Copenhagen, Denmark

Carolina Simó, Laboratory of Foodomics, Institute of Food Science Research (CIAL), National Research Council (CSIC), Madrid, Spain

Manuel Suárez, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Universitat Rovira i Virgili, Tarragona, Spain

Francisco A. Tomás-Barberán, Department of Food Science and Technology, CEBAS-CSIC, Murcia, Spain

Alberto Valdés, Laboratory of Foodomics, Institute of Food Science Research (CIAL), National Research Council (CSIC), Madrid, Spain

Débora Villaño, Department of Food Science and Technology, CEBAS-CSIC, Murcia, Spain
1

FOODOMICS: PRINCIPLES AND APPLICATIONS

ALEJANDRO CIFUENTES

1.1 INTRODUCTION TO FOODOMICS

Research in food science and nutrition 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 rising consumer demands, food and nutrition researchers around the world are facing increasingly complex challenges that require the use of 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 also becoming 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.

The first goal of food science 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 European Union countries (e.g., Regulation EC 258/97 or EN 29000 and subsequent issues), the Nutrition Labeling and Education Act in the USA, and the Montreal Protocol have had a major impact on food laboratories. Consequently, more powerful, cleaner, and cheaper analytical procedures are now required 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.

Currently, there is also a general trend in food science toward the connection between 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) derived from this trend is 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, researchers in food science and nutrition are moving from classical methodologies to more advanced strategies, and usually borrow methods well established in medical, pharmacological, and/or biotechnology research. 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.

In modern food science and nutrition, terms such as nutrigenomics, nutrigenetics, nutritional genomics, transgenics, functional foods, nutraceuticals, genetically modified (GM) foods, microbiomics, toxicogenomics, nutrascriptomics, nutriproteomics, nutrimetabolomics, and systems biology are expanding. This novelty has also brought about some problems related to the poor definition of part of this terminology or their low acceptance, probably due to the difficulty to work in a developing field in which several emerging strategies are frequently put together.

1.1.1 Definition of Foodomics

Although the term Foodomics is being used in different web pages and scientific meetings since 2007 (see e.g., Slater and Wilson, 2007 or Capozzi and Placucci, 2009), Foodomics was for the first time defined in an SCI journal in 2009 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 (Cifuentes, 2009; Herrero et al., 2010, 2012). Thus, Foodomics is not only an useful concept that comprises in a simple and straightforward way all of the emerging terms aforementioned (e.g., nutrigenomics, nutrigenetics, microbiomics, toxicogenomics, nutrascriptomics, nutriproteomics, nutrimetabolomics), but more importantly, Foodomics is a global discipline that includes all the working areas in which food (including nutrition) and advanced omics tools are put together.

A representation of the areas covered by Foodomics and the tools employed can be seen in Figure 1.1. Just to name a few topics that could be addressed by this new discipline, Foodomics would help: (a) to understand the gene-based differences among individuals in response to a specific dietary pattern following nutrigenetic approaches; (b) to understand the biochemical, molecular, and cellular mechanisms that underlie the beneficial or adverse effects of certain bioactive food components
following nutrigenomic approaches; (c) to determine the effect of bioactive food constituents on crucial molecular pathways; (d) to know the identity of genes that are involved in the previous stage to the onset of the disease, and, therefore, possible molecular biomarkers; (e) to establish the global role and functions of gut microbiome, a topic that is expected to open an impressive field of research in the near future; (f) to carry out the investigation on unintended effects in GM crops; (g) to understand the stress adaptation responses of food-borne pathogens to ensure food hygiene, processing, and preservation; (h) to investigate the use of food microorganisms as delivery systems including the impact of gene inactivation and deletion systems; (i) in the comprehensive assessment of food safety, quality, and traceability ideally as a whole; (j) 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; and (k) 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. 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 that are discussed below.
1.1.2 Foodomics Tools

As can be seen in Figure 1.1, Foodomics involves the use of multiple tools to deal with its different subdisciplines and applications. Thus, the use of omics tools such as genomics, epigenomics, transcriptomics, proteomics, and metabolomics is a must in this new discipline. Although a detailed description on these tools is out of the scope of this chapter, some fundamentals about these techniques are provided below.

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 the 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. In mammals, many dietary components, including folate, vitamin B6, vitamin B12, betaine, methionine, and choline, have been linked to changes in DNA methylation. These nutrients can all affect the pathways of one-carbon metabolism that determine the amount of available S-adenosylmethionine, which is the methyl donor for DNA methylation and histone methylation. Although it is too early to apply epigenetic alterations that are induced by dietary ingredients as biomarkers in public health and medicine, research in this area is expected to be boosted by the expanding use of next-generation DNA sequencing technologies. Applications include chromatin immunoprecipitation followed by DNA sequencing (ChIP–seq) to assess the genomic distribution of histone modifications, histone variants and nuclear proteins, and global DNA methylation analysis through the sequencing of bisulphite-converted genomic DNA. Combined with appropriate statistical and bioinformatic tools, these methods will permit the identification of all the loci that are epigenetically altered.

Regarding transcriptomics, the global analysis of gene expression 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 down-regulation of a selected number of genes.

In proteomics, the huge dynamic concentration range of proteins in biological samples causes many detection difficulties because many proteins are below the sensitivity threshold of the most advanced instruments. For this reason, fractionation and subsequent concentration of the proteome is often needed. Besides, the use and development of high-resolving separation techniques as well as highly accurate mass spectrometers is nowadays essential to solve the proteome complexity. Currently, more than a single electrophoretic or chromatographic step is used to separate the thousands of proteins found in a biological sample. This separation step is followed by analysis of the isolated proteins (or peptides) by mass spectrometry (MS) via the so-called “soft ionization” techniques, such as electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI), combined with the everyday
more powerful mass spectrometers. 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. Protein microarrays can be composed by recombinant protein molecules or antibodies immobilized in a high-density format on the surface of a substrate material. There are two major classes of protein micro- (nano-) arrays: analytical and functional protein microarrays, being the antibody-based microarray the most common platform in proteomic studies.

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 (or higher) to picomolar level, making it easy to exceed the linear range of the analytical techniques employed. Metabolites are, in general, the final downstream products of the genome, and reflect most closely the operation of the biological system, its phenotype. The analysis of metabolic patterns and the changes in the metabolism in the nutrition field can be, therefore, very interesting to locate; for example, variations in different metabolic pathways due to the consumption of different compounds in the diet. One of the main challenges in metabolomics is to face the complexity of any metabolome, usually composed by a huge number of compounds of very diverse chemical and physical properties (sugars, amines, amino acids, organic acids, steroids, etc.). Sample preparation is especially important in metabolomics, because the procedure used for metabolite extraction has to be robust and highly reproducible. Sample preparation will depend on the sample type and the targeted metabolites of interest (fingerprinting or profiling approach). Moreover, no single analytical methodology or platform is applicable to detect, quantify, and identify all metabolites in a certain 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 nontargeted strategy that focuses on the study of a group of related metabolites or a specific metabolic pathway. It is one of the basic approaches to phenotyping, because the study of metabolic profiles of a cell gives a more accurate description of a phenotype. Meanwhile, metabolic fingerprinting does not aim to identify all metabolites, but to compare the 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 also become 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 analyze large data lists in an attempt to assemble a summary of the most significant biological aspects. Also, statistical tools are usually applied 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 reducing it with the most relevant ones for biomarkers discovery, etc.

1.2 FOODOMICS APPLICATIONS: CHALLENGES, ADVANTAGES, AND DRAWBACKS

Although there is still a large number of gaps to be filled in our current knowledge on food science and nutrition, the great analytical potential of Foodomics can help resolve many issues and questions related to food safety, traceability, quality, new foods, transgenic foods, functional foods, nutraceuticals, etc.

1.2.1 Food Safety, Quality, and Traceability

Foodomics can help solve some of the new challenges that modern food safety, quality, and traceability have to face. These challenges encompass the multiple analyses of contaminants and allergens; the establishment of more powerful analytical methodologies to guarantee food origin, traceability, and quality; the discovery of biomarkers to detect unsafe products; the capability to detect food safety problems before they grow and affect more consumers; etc.

1.2.2 Transgenic Foods

Although this book includes a chapter devoted to transgenic foods, a brief outline on this topic is given below. Recombinant DNA technology, or genetic engineering, allows selected individual gene sequences to be transferred from an organism into another and also between nonrelated species. Genetic engineering has been used in agriculture and food industries in the past years in order to improve the performance of plant varieties (resistance to plagues, herbicides, and hydric or saline stresses), improve technological properties during storage and processing (firmness of fruits), or improve the sensorial and nutritional properties of food products (starch quality, content of vitamins or essential amino acids). The organisms derived from recombinant DNA technology are termed genetically modified organisms (GMOs). Transgenic food is a food that is derived from or contains GMOs.

The use of genetic engineering in the production of foods is constantly growing since the past years as well as the concern in part of the public opinion. This is due to the increasing impact of this technology in foodstuff production, by one side, and to the continued campaign against GMO crops lead by ecologist organizations, by the other. Claims about the advantages derived from GMO crops include those from
the biotechnology companies and most of the scientific community, stressing the benefits for the agriculture and the food industry and the lack of scientific evidence on any detrimental effects on human health. On the other side, ecologists groups are concerned about the impact of GM plants on human health and on the environment. In this context, most governments have dictated regulations on the use, spreading, and marketing of GMOs, in order to regain the confidence of the consumers. Owing to the complexity that entails the compositional study of a biological system such as GMO, the study of substantial equivalence as well as the detection of any unintended effect should be approached with advanced profiling techniques, with the potential to extend the breadth of comparative analyses. However, there is no single technique currently available to acquire significant amounts of data in a single experimental analysis to detect all compounds found in GMOs or any other organism. In consequence, multiple analytical techniques have to be combined to improve analytical coverage of proteins and metabolites. Namely, the European Food Safety Authority (EFSA) (EFSA, 2006) has recommended the monitoring of the composition, traceability, and quality of these GM foods using advanced analytical techniques including omics techniques to provide a broad profile of these GM foods (Levandi et al., 2008; Garcia-Villaba et al., 2008, 2010; Simó et al., 2010; García-Cañas et al., 2011). The development of new analytical strategies based on Foodomics will provide extraordinary opportunities to increase our understanding about GMOs, including the investigation on unintended effects in GM crops, or the development of the so-called second-generation GM foods. Besides, Foodomics has to deal with the particular difficulties commonly found in food analysis, such as the huge dynamic concentration range of food components as well as the heterogeneity of food matrices and the analytical interferences typically found in these complex matrices.

1.2.3 Foodomics in Nutrition and Health Research

Nowadays, food is investigated not only as a source of energy but also as a potential health promoter. As a result, 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 (García-Cañas et al., 2010). The problem to be resolved 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 signaling molecules that are recognized by specific cellular-sensing mechanisms. 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. Therefore, it is necessary to look at hundreds of test compounds simultaneously and observe the diverse temporal and spatial responses.
Foodomics can be an adequate strategy to investigate the complex issues related to prevention of future diseases and health promotion through food intake. 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 can be 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 1.2. Following this Foodomics strategy, results on the effect of food ingredient(s) at genomic/transcriptomic/proteomic and/or metabolomic levels are obtained, making possible new investigations on food bioactivity and its effect on human health at molecular level. 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. It has been mentioned that it

**FIGURE 1.2** Scheme of an ideal Foodomics strategy to investigate the health benefits from dietary constituents, including methodologies and expected outcomes. Modified from Ibáñez et al. (2012) with permission from Elsevier.
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. In this regard, it is interesting to remark that 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 EFSA about health claims of new foods and ingredients (EFSA, 2010; Gilsenan, 2011). More sound scientific evidences are needed to demonstrate 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 levels, 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. 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 literature in which results from the three expression levels (transcriptomics, proteomics, and metabolomics) are simultaneously presented and merged. Figure 1.3 shows the results from a global Foodomics study on the chemopreventive effect of dietary polyphenols against HT29 colon cancer cells (Ibáñez et al., 2012). Figure 1.3 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. In order to demonstrate all its value, Foodomics still needs to be translated to methods or approaches with medicinal impact, for example, 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 Foodomics, to carry out a comprehensive elucidation of the mechanisms of action of natural compounds, specific nutrients, or diets, in vitro assays or animal models are mainly used because (a) they are genetically homogeneous within a particular assay or animal model and (b) environmental factors can be controlled. Moreover, these assays allow the study of certain tissues that would not be possible to obtain from humans. On the other hand, the main difficulty in the study of diets is the simultaneous presence of a variety of nutrients, with diverse chemical structures, that can have numerous targets with different affinities and specificities. Ideally, the final demonstration on the bioactivity of a given food constituent should be probed by Foodomics based on a global omics study of the biological samples generated during a clinical trial.

It is interesting to mention that there are still rather limited studies on the effect of specific natural compounds, nutrients, or diet on the transcriptome/proteome/metabolome of organisms, tissues, or cells, being the number of review papers on this topic higher than the number of research papers.
FIGURE 1.3  Foodomics identification of the proteins, genes, and metabolites involved in three of the principal biological processes altered in HT29 colon cancer cells after the treatment with rosemary polyphenols. Underlined, down-regulated; Not underlined, up-regulated. Modified from Ibáñez et al., 2012, with permission from Elsevier.