Foodomics:
MS-based Strategies in Modern Food Science and Nutrition

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LIST OF ABBREVIATIONS.

2-DE, two dimensional gel electrophoresis
APCI, atmospheric pressure chemical ionization
BGE, background electrolyte
CE, capillary electrophoresis
CID, collision-induced dissociation
DART, direct analysis in real time
DIGE, differential in-gel electrophoresis
ESI, electrospray interface
FIA, flow-injection analysis
FID, flame ionization detection
FT-ICR, Fourier-transform ion cyclotron resonance
GC, gas chromatography
GCxGC, two-dimensional comprehensive gas chromatography
GMO, genetically modified organism
HCA, hierarchical clustering analysis
HILIC, hydrophilic interaction liquid chromatography
ICA, independent component analysis
ICAT, isotope coded affinity tags
ICP, induced coupled plasma
IRMS, isotope ratio MS
IT, ion trap
LC, liquid chromatography
LCxLC, two-dimensional comprehensive liquid chromatography
LOD, limit of detection
LOQ, limit of quantification
LVI, large-volume injections
MALDI, matrix-assisted laser desorption/ionization
MRL, maximum residue level
MRM, multiple reaction monitoring
MW, molecular weight
NMR, nuclear magnetic resonance
OPLS-DA, orthogonal projection-discriminant analysis
PAGE, polyacrylamide gel electrophoresis
PAH, polycyclic aromatic hydrocarbons
PCA, principal component analysis
PCB, polychlorinated biphenyls
PLE, pressurized liquid extraction
PLS-DA, partial least square discriminant analysis
Q, quadrupole
QqQ, triple quadrupole
QuEChERS, quick, easy, cheap, effective, rugged, and safe
SELDI, surface-enhanced laser desorption/ionization
SFE, supercritical fluid extraction
SPE, solid-phase extraction
SNP, single nucleotide polymorphism
TOF, time-of-flight
UPLC, ultra-performance liquid chromatography

ABSTRACT

Modern research in food science and nutrition is moving from classical methodologies to advanced analytical strategies in which MS-based techniques play a crucial role. In this context, Foodomics has been recently defined as a new discipline that studies food and nutrition domains through the application of advanced omics technologies in which MS techniques are considered indispensable. Applications of Foodomics include the genomic, transcriptomic, proteomic, and/or metabolomic study of foods for compound profiling, authenticity, and/or biomarker-detection related to food quality or safety; the development of new transgenic foods, food contaminants, and whole toxicity studies; new investigations on food bioactivity, food effects on human health, etc. This review work does not intend to provide an exhaustive revision of the many works published so far on food analysis using MS techniques. The aim of the present work is to provide an overview of the different MS-based strategies that have been (or can be) applied in the new field of Foodomics, discussing their advantages and drawbacks. Besides, some ideas about the foreseen development and applications of MS-techniques in this new discipline are also provided.

I. INTRODUCTION TO FOODOMICS.

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, nutritranscriptomics, nutriproteomics, nutrimetabolomics, systems biology, etc., are expanding (Powell, 2007; Rezzi et al., 2007; Rist, Wenzel & Daniel, 2006; Subbiah, 2006; Trujillo, Davis & Milner, 2006). This novelty has also brought about some problems related to the poor definition of part of this terminology or their low acceptance (Ronteltap, van Trijp & Renes, 2007), probably due to the difficulty to work in a developing field in which several emerging strategies are frequently put together.

A. Definition of Foodomics, fundamentals, and tools.

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 in order to improve consumer's well-being, health, and confidence (Cifuentes, 2009; Herrero et

al., 2010). Thus, Foodomics is intended to be not only an useful concept to cover in a simple and straightforward way all of the abovementioned new terminology, but more importantly, it is intended to be a global discipline that includes all of the emerging working areas in which food (including nutrition), advanced analytical techniques (mainly omics tools), and bioinformatics are combined. A representation of the areas covered by Foodomics and its main goals can be seen in Figure 1. For instance, Foodomics would cover the development of new transgenic foods with molecular tools, the genomic/transcriptomic/proteomic and/or metabolomic study of foods for compound profiling/authenticity and/or biomarkers analysis related to food quality, new investigations on food bioactivity and its effect on human health following nutrigenomics and/or nutrigenetics approaches, development of global omics strategies to explore food safety issues, etc. 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 will be discussed below.

Foodomics involves the use of multiple tools to deal with the different applications included in this field. Thus, the use of omics tools such as genomics, transcriptomics, proteomics, and metabolomics is a must in this new discipline. MS-based techniques are crucial for proteomics and metabolomics studies.

Proteomes differ among individuals, cell types, and within the same cell, depending on cell activity and state. An important challenge in proteomic studies is the wide difference in concentration from the most- to the least-abundant proteins (i.e., a dynamic range of 10^{10} has been estimated for protein concentration in serum) that

makes proteome analysis a challenging task. The increasing popularity of proteomics has created a need for quantitative analysis methods. As a result, many different techniques are now available for performing gel-based or gel-free quantitative proteomics. These techniques provide an insight into global protein expression from identification to quantification (Szopinska et al. 2010).

Main sample preparation methods to reduce proteome complexity include, fractionation, depletion, as well as enrichment of low-abundant proteins. Figure 2 shows a scheme of the different strategies that can be followed in order to carry out a proteomic study including “bottom-up”, “shotgun” and ‘‘top-down’’ approaches. MS is used in these strategies as the last analytical step for peptide detection and protein identification. At present, MS represents the most-powerful tool in proteomics because it requires no prior knowledge of the proteins to be identified (Motoyama & Yates, 2008; Yates, Ruse & Nakorchevsky, 2009; Han, Aslnian & Yates, 2008). MS also enables the analysis of proteins and peptides in large-scale and high-throughput modes. Improved mass spectrometers with better sensitivity and superior mass accuracy and resolution aim to identify and quantify complex protein (peptides) mixtures in a single experiment. Main mass analyzers used in proteomics are time-of-flight (TOF), quadrupole (Q), Fourier transform ion cyclotron resonance (FT-ICR), and ion trap (IT), which are usually combined in one mass spectrometer (triple quadrupole (QqQ), Q-IT, Q-TOF, TOF-TOF, IT-FTMS, etc.). Typically, mass spectrometry can cover a dynamic range up to four orders of magnitude.

On the other hand, the 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) (Trujillo, Davis & Milner, 2006). 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 changes in the metabolism in the nutrition field can be, therefore, very interesting to locate; e.g., 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, peptides, organic acids, nucleic acid, or steroids). 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 (Shulaev, 2006). MS and NMR-based technologies are both complementary and, therefore, often used in parallel in metabolomic research. Compared to NMR, MS is a more-sensitive technique; also, MS coupled to GC, LC, or CE allows higher resolution and sensitivity for low-abundance metabolites (Xiayan & Legido-Quigley, 2008; Garcia et al., 2008; Dettmer, Aronov & Hammock, 2007; Issaq et al., 2009). The use of high and ultra-high resolution analyzers (namely, TOF, FTMS, Orbitrap®) is essential to obtain accurate mass measurements for the determination of

elemental compositions of metabolites, and to carry out their tentative identification with databases (Xu et al., 2010; Brown, Kruppa & Dasseux, 2005). On the other hand, MS/MS or MS^n experiments, especially when product ions are analyzed at high resolution (with Q-TOF, TOF-TOF or LTQ-Orbitrap®) provide additional structural information for the identification of metabolites. Although there are three basic approaches used in any metabolomic study (target analysis, metabolic profiling, and metabolic fingerprinting), we will focus in this review on metabolic profiling and metabolic fingerprinting. Metabolic profiling focuses on the analysis of a group of metabolites that are either related to a specific metabolic pathway or a class of compounds. On the other hand, the goal in metabolic fingerprinting is the comparison of patterns of metabolites that change in response to a disease, a treatment, environmental or genetic alterations, etc.

Due to the huge amount of data usually obtained from these omics studies, it has been necessary to develop strategies to convert the complex raw data obtained into useful information. Thus, bioinformatics has become a crucial tool in Foodomics. Over the last years, the use of biological knowledge accumulated in public databases allows one to systematically analyze with bioinformatics large data lists in an attempt to assemble a summary of the most significant biological aspects (Waagmeester, Kelder & Evelo 2008). Also, statistical tools are usually applied; e.g., for exploratory data analysis to determine correlations among samples (which can be caused by either a biological difference or a methodological bias), to discriminate the complete data list and reduce it with the most relevant ones, for biomarkers discovery, etc.

B. New challenges in modern food safety, quality, and traceability studies.

Foodomics can help to solve some of the new challenges that modern food safety, quality, and traceability have to face. These challenges encompass the multiple analysis of contaminants, the establishment of more-powerful analytical methodologies to guarantee food origin and quality, the discovery of biomarkers to detect unsafe products or the capability to detect food safety problems before they grow and affect more consumers, etc. A good example of the application of advanced approaches in food science is the development of transgenic (also called genetically modified, GM) foods in which molecular biology, chemistry, agriculture, and food science are combined in order to adequately develop these new foods. Moreover, the monitoring of the composition, traceability, and quality of these GM foods has been recommended with advanced analytical techniques (EFSA, 2006), including -omics techniques to provide a broad profile of these GM foods (Garcia-Villaba et al., 2010; Levandi et al., 2008; Simó et al., 2010). 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 (García-Villalba et al., 2008; Levandi et al., 2008; Simó et al., 2010). 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.

Moreover, the combined use in Foodomics of advanced analytical methodologies with other more classical approaches, such as toxicity studies, *in-vitro* or *in-vivo* assays, and/or clinical trials, can provide an important added value to the results.

Some examples of these applications that will be discussed below in section 2 of this review work, will pay special attention to the MS-based strategies.

C. Functional foods, nutrition, and health research: A Foodomics approach.

One of the main goals in modern food science and nutrition is to improve our limited understanding of the roles of nutritional compounds at the molecular level (i.e., their interaction with genes, and their subsequent effect on proteins and metabolites). This knowledge should allow the rational design of strategies to manipulate cell functions through diet; that goal is expected to have an extraordinary impact on our health. However, unlike pharmaceuticals, the simultaneous presence of a variety of nutrients, with diverse chemical structures and concentrations, and with numerous targets with different affinities and specificities increases enormously the complexity of the problem. The development of genomics, transcriptomics, proteomics, and metabolomics provides extraordinary opportunities to increase our understanding in regards to this huge variability addressed by Foodomics. A detailed description on genomics and transcriptomics is out of the scope of this paper (readers interested on these topics can find useful information elsewhere (Dettmer, Aronov & Hammock, 2007; García-Cañas et al., 2010; Griffiths & Wang, 2009; Raqib & Cravioto, 2009), which will focus on MS-based strategies in the new field of Foodomics with special emphasis in proteomics and metabolomics applications.

II. MASS SPECTROMETRY IN FOODOMICS.

A. Food safety, quality, and traceability with MS-based “omics” approaches.

1. Detection of exogenous contaminants in food.

Food safety is today a challenging field in which modern analytical chemistry must provide accurate, precise, and robust methods to determine any harmful compounds or organisms that might be present in food at very low concentrations. The evolution of MS and the application of Foodomics technologies have a very significant impact on this field, and improve even further the limits demanded by food safety legislation. A clear example of this trend is the continuous development of multi-residue methods for the sensitive determination of contaminants in food, mainly pesticides and antimicrobials. The employment of these classes of compounds is common practice in agriculture and farming to prevent possible issues that might threaten the correct growth of crops and animals. However, in order to limit and control the use of these compounds, and to consequently protect the health of the consumers, the legislation of different countries imposes strict maximum residue levels (MRLs) – defined as the maximum amount of a particular compound that might reach the final food product (Bohm, Stachel & Gowik, 2009). These limits are established so that the employment of these compounds does not pose a risk for human health, whereas the use of some of them is strictly forbidden. The use of MS coupled to other analytical techniques, mainly separation methods, allows the simultaneous and sensitive determination of these compounds in food matrices. In Table 1, some representative researches recently published on the simultaneous analysis of more than 30 pesticides and/or antimicrobials are summarized. Table 1 shows that modern mass spectrometers allow the simultaneous and sensitive quantification of diverse groups of compounds in different food matrices. Several advanced separation techniques are typically coupled to MS instruments to develop this kind of applications, as shown in Table 1. Independently from the

analytical tool selected, MS/MS analyses are performed in which two product-ions are usually selected for each precursor-ion for these determinations (in Comission decision 2002/657/EC). Multiple reaction monitoring (MRM) is the most frequently employed mode to selectively measure the intensity of the quantifier ion. The other product-ion, normally called a qualifier ion, confirms the positive identification of the contaminant. Besides the ionization source settings, the collision-induced dissociation (CID) parameters for the in-source CID fragmentation are of great importance, and should be carefully optimized, because they have a strong influence on the sensitivity. Besides, in order to acquire precise and reproducible results, the calibration performed must be closely evaluated. Internal and external standard calibrations have both been applied. Whereas the contaminants must be usually extracted from the "real" sample in a step previous to their analysis, matrix-matched calibrations are frequently used (Economou et al., 2009). This kind of procedure facilitates the selection of the calibration method by observing statistically whether the sample matrix positively or negatively influences the results. Some matrices could have a negative effect in the ionization of the analytes (an inhibition effect), whereas others might promote the ionization of the studied contaminants (Carretero et al., 2008).

Among the coupled analytical techniques employed for these applications, the combination of liquid chromatography (LC) with electrospray interface (ESI) and triple quadrupole analyzers is the most frequently used. This technique has been successfully employed to determine pesticides in fruits (Wong et al., 2010), vegetables (Chung & Chan, 2010), wines (Economou et al., 2009), milk (Dagnac et al., 2009), or meat (Carretero, Blasco & Pico, 2008), for example. Solid-phase extraction or QuEChERS (abbreviation of Quick, Easy, Cheap, Effective, Rugged, and Safe) are the most-

employed sample pretreatment methods. With LC-MS/MS, values of LOQs as low as few μg Kg^{-1} are generally reached; also, the analyses are relatively fast. For instance, 58 antibiotics were analyzed in milk in less than 15 min (Gaugain-Juhel et al., 2009). However, the selective detection of the triple quadrupole analyzers allows the accurate analysis of incompletely separated compounds. In fact, in almost the same analysis time (i.e., 14 min), 191 pesticides residues were determined from different fruits (Wong et al., 2010a). In order to further speed-up these separations, nowadays, short columns with smaller particle diameters are also employed, to produce separations at ultra-high pressures in UPLC. With this equipment, more than 100 pesticides were analyzed in strawberry samples in less than 5 min (Taylor et al., 2008). Gas chromatography (GC) is other technique routinely coupled to triple quadrupole analyzers to determine food contaminants. GC presents a series of advantages over LC, such as lower use of organic solvents or higher efficiency; however, the separations are usually slower and the sensitivity might be compromised. Nevertheless, GC-MS/MS methods of comparable high throughput have been also developed; for instance, for the separation and quantification of more than 160 pesticides residues in vegetables (Wong et al., 2010b), to attain limits of detection similar to those obtained with LC-MS/MS. Besides, other approaches such as large-volume injections (LVI) can be employed to further improve the GC-MS sensitivity (Xu et al., 2009). Although some GC-MS research has employed the SIM mode to detect and quantify the contaminants (mainly due to the higher accessibility to a single quadrupole instrument), it has been demonstrated how the use of GC-MS/MS significantly increases the specificity, sensitivity, and reliability of the method (Wong et al., 2010b). Also recently, comprehensive two-dimensional GC (GCxGC) has been used to quantitatively determine more than 100 pesticides by coupling GCxGC to TOF-MS (Van der Lee et al., 2008). This technique is gaining

attention due to its capability to characterize unknown samples. However, quantitative analyses have not been reported extensively, mainly due to problems related to data handling. Nevertheless, this technique can quantify multiple pesticides with an LOQ at μg kg^{-1} levels (Van der Lee et al., 2008). Although conventional GC-MS is a more affordable technique, the coupling of two different separation mechanisms in GCxGC-MS through a modulator allows increasing the separation power, while introducing different separation mechanisms. Besides, a characteristic of GCxGC-MS compared to GC-MS is its increased signal-to-noise ratio thanks to the refocus of a chromatographic band produced in the modulator and its subsequent release to the second dimension separation. In Figure 3, the three-dimensional and contour plots that correspond to a separation of a high number of pesticides with GCxGC-TOF-MS is shown. The increased separation power of this technique as well as an appropriate modulation between the two dimensions might improve the detection capabilities of the mass spectrometers and enhance the sensitivity. Similar approaches can also be employed for the determination of other contaminants in food, e.g., from environment or compounds that might migrate from packing materials, as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines or phthalates (Malik, Blasco & Pico, 2010).

2. Detection of food allergens.

The detection of food allergens is a hot topic in the food safety field. Food allergens are naturally present in some foods, and might induce adverse reactions in susceptible individuals. The interest on food allergies keeps therefore increasing, while a complete cure for the different food allergies is not yet available. Thus, allergic patients are forced to avoid the consumption of the allergen, even in very small quantities. This fact has led

to tighter legislation by food control agencies (FDA, EFSA, etc), in order to make clear in the food labels the presence and/or amount of the most-common food allergens. A relatively high number of proteins have been identified as food allergens in different food products, such as dairy products, eggs, soybeans, peanuts, cereals or fish, for instance. Consequently, proteomics has become a very useful tool for the identification of allergens in food products. In this regard, MS is widely used for the sensitive detection and identification of allergen food proteins. Up to now, the bottom-up proteomics strategy is the most widely used method for the detection of food allergens. As mentioned above, this strategy includes protein digestion in order to produce a set of peptides derived from the different proteins contained in the sample and their identification as a part of a particular protein. This approach has been used, for instance, to combine capillary LC with Q-TOF MS in order to select a series of peptides as markers for the presence of the three major peanut allergens (Ara-h1, Ara-h2, and Ara-h3) in food products independently of the use as raw or roasted peanuts (Chaissaigne, Norgaard & Van Hengel, 2007). This fact was shown to be very important because roasting significantly affected the detectability of a large number of ions derived from these allergens. Therefore, the development of this strategy allows the detection and identification of traces of peanut allergens in products that might non-intentionally contain peanuts. In fact, similar approaches have demonstrated their capabilities to detect concentrations of peanut allergen proteins as low as 1 $\mu g\ g^{-1}$ in complex food products, such as rice crispy/chocolate-based snacks (Careri et al., 2007). On the other hand, the use of MS to characterize intact allergen proteins previously detected with immunoblotting with sera from allergic patients has been also extensively carried out. This approach was recently proved to be useful to determine that the α'- and β-subunits of β-conglycinin were also potential soybean allergens (Krishnan et al., 2009). These

two proteins subunits were identified with MALDI-TOF MS after immunoblotting soybean proteins with sera from soybean-allergic patients. A similar approach was followed to identify allergens in cow milk proteins (Natale et al., 2004). Nonetheless, in this case, after the identification of the allergens with immunoblotting, they were separated with 2-DE, and the cow milk proteins and their isoforms were characterized with MALDI-TOF MS (Natale et al., 2004). The combination of immunoassays with MS has also shown great capabilities for the characterization of food allergens. Ig-E immunoblotting was used to reveal potential allergens in tomato fruits and seeds whereas a multidimensional protein fractionation strategy and LC-MS/MS were used for the precise molecular characterization of the allergens (Bassler et al., 2009). In the study of food allergies, surface-enhanced laser desorption/ionization (SELDI) microarrays have been demonstrated to be effective tools for the detection of new food allergens, as effective as traditional Western blot, but faster. This procedure was employed to identify and characterize allergens in banana (Hsieh et al., 2002). Nevertheless it is also useful to detect known allergens, such as lysozyme in cheeses (Dragoni et al., 2010).

3. Detection of pathogens and toxins.

Detection of pathogens, toxins, and sub-products in food spoiled by microorganisms is a relevant aspect of food safety. GC-MS has been used to profile metabolites from food products to identify volatile compounds related to a particular microbial contamination. After this metabolomic approach, volatiles were identified in contaminated meat samples that were generated only when a particular microorganism was present (Ercolini et al., 2009). More than 100 metabolites were correctly identified in the different contaminated meat samples, and their relationships were studied. It was also

found that, not only some volatiles appeared as a result of their release as a consequence of the growth of a particular bacteria, but also that the volatile compounds profile changed significantly between the contaminated and control meat samples (Ercolini et al., 2009). This basic approach can be also combined with chemometric strategies in order to correctly analyze the results obtained from the metabolic profiling. In fact, principal component analysis (PCA) was used to identify important regions in the GC-MS chromatogram that resulted from the profile of volatile organic compounds from natural spoiled pork and pork contaminated with *Salmonella typhimurium* (Xu et al., 2010). Once the important regions in the chromatogram were identified, peak deconvolution was applied in order to increase the certainty of the peak identification. Thanks to this combination of profiling plus chemometrics, a clear distinction between the two groups of samples was possible (Xu et al., 2010). MS has been also used to directly identify microorganisms or even strains that might contaminate food, through the application of proteomics tools. MALDI-TOF-MS has been applied to identify and characterize low molecular weight proteins extracted from intact bacterial cells (Bohme et al., 2010) or even ribosomal proteins (Barbuddhee et al., 2008). From reference MS fingerprints (from 2000 to 10000 Da), it was possible to differentiate among the different bacterial species and genera under study (Bohme et al., 2010). Interestingly, the statistical study of the phylo-proteomic relationships based on the MS data provided the same clustering than the phylogenetic analysis based on the 16S rRNA gene; those data demonstrated the usefulness and applicability of the procedure. Besides, the comparison of the reference profiles and the profiles obtained from seafood samples allowed the adequate application of this methodology to identify unknown bacterial strains (Bohme et al., 2010). In Figure 4, examples of the used reference profiles are depicted. Pathogens have also been identified based on the characterization of

distinctive peptides contained in proteolytic digests with a separation technique (CE) coupled to tandem MS (Hu et al., 2006). The determination of the amount of microorganisms present in a sample used ICP-MS with an immunoassay with antibody-conjugated gold nanoparticles (Li et al., 2010). Quantitative determination of *E. coli* used the direct determination of Au at *m/z* 197, thanks to the previous interaction between the antibody-conjugated gold nanoparticles and *Escherichia coli* O157:H7 cells (Li et al. 2010).

Similar strategies to those previously described to detect contaminants are usually employed to detect toxins in foods. These strategies include the use of an extraction mechanism, followed by a high-resolution separation step coupled to MS. The QuEChERS method has been frequently applied for the simultaneous extraction and analysis of relatively wide groups of toxins (Rasmussen et al., 2010; Zachariasova et al., 2010). Triple quadrupole analyzers provide an LOD of few $\mu g\ kg^{-1}$ (Rasmussenet al., 2010), whereas UPLC speeds-up the separation method (Zachariasova et al., 2010). Moreover, the coupling of UPLC to high resolution mass spectrometers, such as orbitrap, significantly enhanced the accuracy of the determination, and maintained comparable LOD to those obtained with a TOF-MS analyzer. However, the use of the orbitrap minimized the sample treatment and handling, and increased the sample throughput (Zachariasova et al., 2010). It is interesting to mention that, even if the mass accuracy provided by orbitrap analyzers is one order of magnitude higher than most common tandem mass analyzers, it has not been possible so far to reach the number of identification points required for the current EU legislation for the analysis of mycotoxins (Comission decision 2002/657/EC), because accuracy is not considered in the identification points system implemented by the EU (Comission decision

2002/657/EC). Thus, unless the existing criteria evolve towards higher mass accuracy, the full potential of these MS instruments will not be completely helpful for regulatory food control.

4. Food quality and geographical origin assessment.

In terms of food quality, metabolite fingerprinting with separation techniques coupled to MS provide valuable information on the precise composition of food products that can be directly correlated to their quality. Comprehensive GC-MS fingerprinted the volatile fraction of roasted hazelnuts (Cordero et al., 2010), whereas the corresponding volatile fingerprint of beer was obtained with GC-TOF-MS (Cajka et al., 2010). LC-MS was used to profile the flavonols and anthocyanins in grapes (Mattivi et al., 2006), and direct-flow injection MS-based profiling has also been used for beer (Araujo et al. 2005) and potato samples (Beckmann et al., 2007). MS-based metabolite profiling determined the changes during germination of rice (Shu et al., 2008), characterized milks according to their production conditions (Fernandez et al., 2003), assessed the possible impact of the different wheat-farming systems (Zorb et al., 2006), as well as to reveal the effects of pre-storage UV irradiation in apples that can be also correlated to their quality (Rudell, Mettheis & Curry, 2008).

A good example of the use of Foodomics for food quality issues is the development of food metabolomes through the implementation of specific metabolites databases. Tomato metabolome has been collected as a database with the information provided by LC-MS (Moco et al., 2006). The human milk glycome was also determined by combining LC-MS with stand-alone high-resolution MS (MALDI-FT-ICR-MS) to

identify with higher accuracy the oligosaccharides contained in human milk (Ninonuevo et al., 2006).

A quality characteristic that makes so valuable the olive oil is its triacylglycerol composition, and therefore, fatty acid composition. Direct analysis in real time (DART) coupled to TOF-MS was employed to obtain the comprehensive profiling of triacylglycerols from olive oil (Vaclavik et al., 2009). The implementation of this methodology, together with linear discriminant analysis, not only differentiated among diverse olive oil-related products (extra virgin olive oil, olive oil pomace, olive oil), but also emerged as a good alternative to reveal extra virgin olive oil adulteration with hazelnut oil – a commonly employed adulterant (Vaclavik et al., 2009). On the other hand, the determination of fatty acids from olive oil with direct infusion MS also permitted the prediction of the genetic variety used to obtain different extra virgin olive oils (Lerma-Garcia et al., 2008; Gomez-Ariza, Arias-Borego & Garcia-Barrera, 2006). Considering the low polarity of these compounds, MALDI-TOF MS have been also demonstrated as a very useful analytical approach for lipidomics studies (Fuchs & Schiller, 2009). Likewise, the multiple detection of other food components, for instance polyphenols, based on the use of direct MS can be employed to establish metabolite fingerprints useful for food quality avoiding the coupling to a separation technique (Fulcrand et al., 2008).

Proteomics approaches have been also applied to assess food quality because protein profiling can give useful information on food composition, origin, or adulteration (Carbonaro, 2004). Wine proteins are very important to the wine quality because they affect taste, clarity, and stability. To better-know the proteins present in

wine, as well as their possible functions, an LC-MS/MS method was used (Kwon, 2004). The protein profiling of a white wine revealed the existence of 20 proteins in the samples. Interestingly, only 5 of them were directly derived from the grape. Twelve additional proteins belonged to yeast, two from bacteria, and one from fungi. The results indicated the possibility of contaminations due to infections on the vineyard or even improper handing during the harvest and winemaking procedures (Kwon, 2004). Moreover, this type of applications might open new possibilities to detect adulterations if the protein profile could be correlated to the corresponding grape variety. The use of multivariate analysis was useful to analyze the data from the MALDI-TOF-MS analysis of different wheat proteins previously separated with 2-D PAGE (Gottlieb et al., 2002). Gliadin is a protein from the wheat gluten complex that determines whether the crop can be employed or not in breadmaking. The PCA of the MS proteomic data differentiated wheat varieties according to this quality parameter (Gottlieb et al., 2002); that study demonstrated the usefulness of proteomic approaches combined with chemometrics for food quality. Also, peptides naturally present in some foods are of importance to food quality, considering the bioactive activities that might have, as well as those that could be formed during the digestion. Different proteomic studies have been developed to identify this kind of bioactive peptides (Gomez-Ruiz et al., 2006; Gagriaire et al., 2009).

Geographical origin is one of the most-important quality parameters for some foods. The high added value that a particular origin might have compared to others has brought about the protected denomination of origin of some foods. This importance of the foods' origin has caused the appearance of frauds or adulterations with similar less-valuable products. In this sense, the assessment of the origin authenticity of food products is of great importance for food quality. However, one of the most important

difficulties to carry out geographical certification is the appropriate selection of suitable markers. MS-based techniques combined with statistical analysis can effectively help to solve this limitation. One of the most-employed approaches is ICP-MS for the elemental fingerprint combined with chemometrics, mainly multivariate analysis techniques (i.e., principal component, canonical discriminant, linear discriminant analysis) for the classification of samples with different geographical origin. This strategy has been applied, for instance, to assess the authenticity of paprika (Brunner et al., 2010), olive oil (Benincasa et al., 2007), honey (Chudzinska & Baralkiewicz, 2010) and tomato products (Lo Feudo et al., 2010). Other possibility for geographical discrimination of foods seems to rely on the fingerprinting of metabolites. The determination of the volatiles present in honey (Mannas & Altug, 2007) or coffee (Risicevic, Carasek & Pawliszyn, 2008) are two examples of this kind of applications. In fact, the combination of head space, solid phase microextraction, and GC-TOF-MS isolated and identified more than 100 volatile compounds in coffee samples, with different experimental designs to determine the extraction conditions that provided a higher number of volatiles extracted. The complete subsequent analysis, in 8 min, attained semi-quantitative results that were submitted to PCA statistical evaluation to establish the geographical discriminations (Risticevic, Carasek & Pawliszyn, 2008).

Another metabolite-based option with MS, is the use of stable isotope ratio MS (IRMS). This technique detects small differences in stable isotopes that can be correlated to different origins or even to adulterations of products. Besides, the combination of this technique with GC allows the isotopic analysis of each separated compound. The effectiveness of this technique to detect differences in the isotopic

carbon composition for the authentication of mandarin essential oils has been recently demonstrated (Schiplilliti et al., 2010).

The exhaustive study of food proteins and their relationships is also useful to detect food-adulteration. Protein profiling with MS can search for biomarkers that permit the characterization of food samples according to their origin. Levels of 1049 proteins were recorded in organic and conventional wheat of different seasons (Zorb, Betsche & Langerkamper, 2009). After statistical analysis, 25 proteins possessed different levels in the two wheat classes. After considering the seasonal influence, 16 were selected as diagnostic proteins, and were identified with MALDI-TOF-MS after 2-DE. The determination of these 16 proteins could authenticate organic wheat (Zorb, Betsche & Langerkamper, 2009). A quite similar approach with 2-DE and MALDI-TOF-MS was applied to fish (Mazzeo et al., 2010) and shrimp authentication (Ortea, Cañas & Gallardo, 2009). In both cases, precise biomarkers were found. In other interesting research, Wang et al. developed a fast method for the fingerprinting and barcoding of honey proteins with a MALDI-TOF mass spectrometer (Wang et al., 2009). Starting from the information collected by MS, protein fingerprints were generated that were translated into a database library of spectral barcodes. Figure 5 shows the procedure. Once the library was acquired, the authentication confirmation was performed in honey samples through pattern matching.

The study of proteins can be also useful to detect adulterations in food products. The aim of these applications usually is the detection of proteins that are not a part of the proteome of the studied product. An example of this approach was the development of an untargeted LC-QTOF-MS method for the proteins analysis in skimmed milk

powder (Cordawener et al., 2009). The significantly lower price of other vegetable-protein preparations induces the partial adulteration of the dairy product with soy or pea proteins. It was demonstrated that this procedure detected the presence of adulterations in the milk powder based on differential peptide profiling.

5. Food traceability.

Foodomics plays an important role for food traceability in which MS-based metabolomics and proteomics are applied. As an example, the profiling of metabolites can be used not only to determine the origin, but also to obtain the traceability of a given food, that is, to precisely know all the different manufacture steps to which a particular food has been submitted. Thus, powerful separation techniques such as comprehensive 2D GC has been combined with high-resolution MS (TOF-MS) to profile monoterpenoids in grapes (Rocha et al., 2007). This profiling allowed knowing the precise monoterpenoid composition of the different varieties of grapes, and their application allowed the traceability of the products directly derived from these grapes, such as must and wines. A similar strategy was devised aimed to the correct origin traceability of honey samples (Cjaka et al., 2009). In that case, artificial neural networks were used in the chemometric calculations in order to obtain a correct correlation between the volatiles profile and the origin of the honey.

The attainment of peptide profiles with MALDI-TOF-MS has been proposed as another useful tool for food traceability (Chambery et al., 2009). Barcodes derived from these MS data were suggested for the adequate establishment of food traceability.

B. MS-based methodologies to develop and characterize transgenic foods.

The rapid progress of recombinant DNA technology (or genetic engineering) has opened new prospects in the development of novel foods and food ingredients (Petit et al., 2007). Recombinant DNA allows selected individual gene sequences to be transferred from an organism into another and also between non-related species. The organisms derived from recombinant DNA technology are termed genetically modified organisms (GMOs). A transgenic food is a food that is derived from or contains GMOs.

Owing to the complexity that entails the compositional study of a biological system such as GMO, the study of substantial equivalence (OECD, 1993) as well as the detection of any unintended effects (Ioset et al., 2007) should be approached with advanced profiling techniques, with the potential to extend the breadth of comparative analyses (EFSA, 2006). 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 (Saito & Matsuda, 2010). In consequence, multiple analytical techniques have to be combined to improve analytical coverage of proteins and metabolites.

1. Proteomics.

MS-based proteomic analysis has become a key technology for the characterization of proteins and peptides in transgenic food and food ingredients. Based on the so-called bottom-up approach, two-dimensional gel electrophoresis (2-DE), followed with image analysis, and MS (typically MALDI-TOF-MS) or different variants of LC-MS, is the most commonly used analytical methodology to study differentially expressed proteins in GMOs (García-Cañas et al., in press). 2-DE provides the highest protein-resolution capacity with a low-instrumentation cost. This strategy has been used to compare

protein profiles of GM tomatoes (Corpillo et al., 2004; Di Carli et al., 2009), maize (Albo et al., 2007; Zolla et al., 2008), wheat (Di Luccia et al., 2005; Scossa et al., 2008), *Arabidopsis thaliana* (Ren et al., 2009, Ruebelt et al., 2006a; 2006b; 2006c), and potatoes (Careri et al., 2003; Lehesranta et al., 2005) versus their corresponding unmodified lines.

In 2-DE, besides the technical limitations to separate highly hydrophobic, extreme isoelectric point, or high molecular weight (MW) proteins, one of the major sources of error is the gel-to-gel variation that makes difficult an exact match of spots in the image-analysis process. Different approaches, such as the use of multi-gel systems, have been investigated to improve gel-to-gel reproducibility (Zhan & Desiderio, 2003). Recently, Brandao et al. (Brandao, Barbosa & Arruda, 2010) used a strictly controlled routine for image analysis of 2-D gels for the comparative analysis of GM soybean proteome and the corresponding non-modified soybean line. Eight out of ten protein spots that showed changes in expression were characterized and identified with MALDI-QTOF-MS as storage proteins, actin, and a sucrose-binding protein. Also, DIGE can help to circumvent the gel-to-gel variance problem for comparative proteomics by loading different samples labeled with ultrahigh-sensitive fluorescent dyes, typically Cy5 and Cy3, in the same gel (Timms & Cramer, 2008). Islam et al. applied DIGE to compare the proteomes of wild-type cultivars with two GM pea lines that express α-amylase inhibitor from the common bean (Islam et al., 2009). Proteins from individual excised spots were digested with trypsin, and the peptides were analyzed with LC-ESI-QTOF-MS. Approximately 600 proteins with MW ranging from 15 to 100 kDa and pIs between 3 and 10 were resolved in the gels. In that study, the gel images for the analysis of one of the GM peas displayed 66 spots that showed

significant changes. The identification of some of the spots revealed alterations in seed storage proteins.

An LC-ESI-IT-MS has been recently developed to characterize maize cultivars from different origins (García-López, Garcia-Cañas & Marina, 2009). The analyses revealed MS spectral signals that seemed to be characteristic of cultivars with a same geographical origin. A CE-ESI-MS was applied for the analysis of an intact zein-protein fraction from three different GM maize cultivars and their corresponding isogenic lines (Erny et al., 2008). A comparative study of two different mass analyzers, namely, TOF and IT, was carried out. Results showed similar sensitivity and repeatability for both instruments; however, CE-ESI-TOF-MS provided a better number of identified proteins. A comparison of the protein profiles obtained with CE-ESI-TOF-MS did not show any significant differences between the GM lines and their non-modified counterpart. Recently, a novel CE-ESI-TOF-MS profiling method, based on shotgun-proteomics strategy, was developed to investigate any unintended effects in GM soybeans (Simó et al., 2010). With this method, 151 peptides were obtained for each soybean line (see Figure 6); however, no differences between GM soybean and its conventional counterpart were found.

2. Metabolomics.

The use of GC-MS to study the metabolome of GMOs has been one of the most popular strategies reported in the literature because the technique provides high separation efficiency and reproducibility, and it allows the analysis of primary metabolites such as amino acids, organic acids, and sugars with chemical derivatization. In one of the first works on this topic, Roessner et al. applied GC coupled to a quadrupole mass

spectrometer to characterize the metabolic composition of transgenic potato tubers with modified sugar or starch metabolism (Roessner et al., 2000). Identification of the compounds was carried out by spectra comparison with commercial mass spectrum libraries plus the injection of standard compounds. Quantitation of metabolites based on this methodology provided data comparable to those obtained using enzymatically linked photometric assays or HPLC analysis. The identification of 77 out of 150 compounds detected with GC-MS provided valuable information on the altered metabolic pathways, and unexpected changes in the levels of some compounds in the transgenic tubers. In a separate report, the GC-MS analysis of GM potato tubers with altered sucrose catabolism indicated an increased level of amino acids (Roessner, Willmitzer & Fernie, 2001). The suitability of GC-MS in combination with data-mining tools (e.g., PCA and hierarchical clustering) to discover differences that enable the discrimination of the transgenic potato and tomato lines from the respective non-modified lines, has been also demonstrated in further researches (Roessner et al., 2001; Roessner-Tunali et al., 2003). GC-MS is a valuable tool to profile aroma compounds in transgenic fruits and vegetables. Malowicki, Martin & Qian applied GC-MS to investigate the volatile fraction of GM raspberries with added resistance to virus attack not observing any significant differences between the GM line and the wild-type (Malowicki, Martin & Qian, 2008). Similarly, the qualitative and quantitative composition of the aroma among four lines of GM cucumber that overexpress thaumatin II gene and their unmodified lines were also investigated with GC-MS (Zawirska-Wojtasiak et al., 2009).

The combined use of supercritical fluid extraction (SFE) and GC-MS has been used to investigate any unintended effects in GMOs (Bernal et al., 2005). Profiling and

quantification of the extracts detected differences in the amino acid content by the comparison of five different transgenic lines with their corresponding isogenic lines grown under the same conditions. In another research, the relative concentrations of 44 fatty acids (saturated and unsaturated fatty acids, including cis/trans isomers and minor fatty acids) of GM maize and soybean seeds have been compared with GC-MS with those of isogenic lines grown in the same conditions (Jimenez et al., 2009).

Catchpole et al. used two MS-based techniques to obtain complementary data on the compositional similarities/differences between transgenic potato designed to contain high levels of inulin-type fructans and its conventional counterpart (Catchpole et al., 2005). Initially, flow-injection analysis (FIA) ESI-MS was used to analyze 600 potato extracts. Data sets were analyzed with PCA to identify top-ranking ions for genotype identification. Further GC-TOF-MS profiling of more than 2000 tuber samples provided complementary data that covered 242 individual metabolites (90 positively identified, 89 assigned to a specific metabolite class, and 73 unknown). In a further research, Zhou et al. have used GC-MS exclusively used to identify certain important compounds after GC- flame ionization detection (FID) profiling of insect-resistant GM rice (Zhou et al., 2009).

Shin et al. used LC-MS to study transgenic rice with altered production of various flavonoids (Shin et al., 2006). Similarly, the study of flavonoid profiles in pathogen-resistant GM wheat was investigated with LC-MS (Ioset et al., 2007). In this case, flavonoids were extracted with SPE and analyzed with LC-IT-MS with two different ionization sources-ESI and APCI. Additional LC-MS/MS experiments differentiated between C-glycoside flavonoids and O-glycoside analogs. A novel LC-

MS method has been developed for the profiling of stilbenes, a specific class of polyphenols, in transgenic tomato that overexpress a grapevine gene that encoded the enzyme stilbene synthase (Nicoletti et al., 2007). With this methodology, differences in the concentration of rutin, naringenin, and chlorogenic acid were detected when transgenic tomatoes were compared to the control tomato lines. The combined use of LC-MS with GC-MS improved the description of the metabolome status of GMOs. In this regard, differences in some phenolic compounds and volatile secondary metabolites that belong to the classes of monoterpenes, C12-norisoprenoids, and shikimates were detected with LC-ESI-IT-MS and GC-MS for the comparative analysis of GM grapevine lines with the unmodified control (Tesniere et al., 2006).

Although reversed-phase is the most frequent mode used in LC-MS metabolite profiling in GMO analysis, other suitable modes are useful. For example, the levels of the major carbon metabolites in transgenic rice that overexpress ADP-glucose pyrophosphorylase have been determined with a HILIC (hydrophilic interaction liquid chromatography) phase for the separation in LC-ESI-MS/MS (Nagai et al., 2009). In a recent paper, Matsuda et al. analyzed different plant tissues of GM rice with LC-ESI-Q-MS. Metabolic profile data were analyzed with three different statistical methods (i.e., independent component analysis (ICA), correlation analysis, and Student's t-test) to determine the peaks that characterize the difference between GM rice and the unmodified counterpart (Matsuda et al., 2010). Complementary LC-MS/MS analysis identified 26 peaks selected after the statistical treatment. Results obtained in the study also indicated that the concentration of Trp changed in a time-dependent manner to show a tissue-dependent profile of accumulation.

The potential of CE-MS for metabolic profiling of GMOs has already been demonstrated to study GM rice (Takahashi et al., 2006). Identification of chemical compounds was performed by comparison of their *m/z* and migration times with standard metabolites. Novel methods, based on CE-MS for metabolite profiling of GM maize and soybean, have been developed recently (Levandi et al., 2008; Garcia-Villalba et al., 2008). Thus, CE-ESI-TOF-MS was used to determine statistically significant differences in the metabolic profile of varieties of conventional and insect-resistant GM maize (Levandi et al., 2008). A similar CE-ESI-TOF-MS methodology was developed for the comparative analysis of metabolic profiles from transgenic soybean (glyphosate resistant) and its corresponding unmodified parental line (García-Villalba et al., 2008). In that study, over 45 different metabolites, including isoflavones, amino acids, and carboxylic acids were identified. Recently, Giuffrida et al. developed a chiral CE-ESI-TOF-MS method to study differences in the chiral amino acid profile among varieties of conventional and transgenic soybean modified to be tolerant to glyphosate herbicide (Giuffrida et al., 2009). In that research, the obtained D/L-amino acid profiles were very similar for conventional and GM soybean.

FT-ICR-MS has already been used as a powerful analytical platform for metabolomic studies in GMOs (Aharoni et al., 2002; Takahashi et al., 2005; Mungur et al., 2005). Owing to its excellent mass resolution (greater than 100,000) and accuracy (sub-ppm), FT-ICR-MS enables molecular formula determination from a vast number of different compounds to be determined in direct-infusion analyses of complex samples without any previous chromatographic or electrophoretic separation and/or derivatization reaction. However, poor ionization of interesting analytes might occur due to matrix effects during direct infusion. In addition, FT-ICR-MS offers moderate

sensitivity and quantitative capabilities. In a recent research, CE-TOF-MS and FT-ICR-MS were used for the metabolomic profiling of six varieties of maize, three GM insect-resistant lines, and their corresponding isogenic lines (Leon et al., 2009). The FT-ICR-MS data obtained in positive and negative ESI mode were both uploaded into a MassTRIX server (Suhre & Schmitt-Kopplin, 2008) in order to identify maize-specific metabolites annotated in the KEGG (Kyoto encyclopedia of genes and genomes) database. Despite the mentioned good mass resolution and accuracy of the technique, certain compounds could not be unequivocally identified, because FT-ICR-MS cannot differentiate isomers that have the same molecular formula, so that migration time, electrophoretical mobilities, and *m/z* values provided by CE-TOF-MS were used to confirm the identity of various compounds.

C. Foodomics in nutrition and health research. MS-based "omics" approaches in Nutrigenomics, Nutriproteomics, and Nutrimetabolomics.

Nutrigenomics is a branch of Foodomics that focuses on the study of the effects of foods and food constituents on gene expression. Nutrigenomics studies the impact of specific nutrients on health through the expression of genetic information by the integration of "omics" technologies such as transcriptomics, proteomics, and metabolomics. MS-based techniques were applied for proteomics and metabolomics, whereas transcriptomics studies the mRNA expression of genes with microarray technology and techniques based on DNA sequencing.

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 (i) they are genetically homogeneous within a

particular assay or animal model, and (ii) environmental factors can be controlled. Moreover, these assays allow the study of certain tissues that would be not possible to obtain from humans. On the other hand, the main difficulty on 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.

From a proteomics point of view, in order to glean an insight on the effect of specific natural compounds, nutrients, or diet on the proteome of organisms, tissues, or cells, comparative proteomics strategies are mainly used. Most of them are based on a bottom-up proteomic approach; more precisely, in a combination of classical 2-DE separation of proteins and MS detection of the in-gel digested proteins. It is interesting to mention that there are still rather limited studies on the effect of specific natural compounds, nutrients, or diet on the proteome of organisms, tissues, or cells; the number of review papers on this topic is higher than the number of research papers (de Roos & McArdle, 2008). Table 2 shows some representative Nutrigenomics applications that use MS-based proteomics. For instance, dietary antioxidants have been studied as candidate chemopreventive agents against carcinogenesis and inhibition of tumor progression. Proteomics is a key tool to explore the molecular mechanisms involved in their anticancer activity. In a recent research, dietary supplementation with three combined micronutrients (vitamin E, selenium, and lycopene) was studied by Cervi et al. of which vitamin E and lycopene have recognized antioxidant activity in mice (Cervi et al., 2010). In this study a first expression difference mapping using the

purified peptide-containing fraction from mice plasma was carried out using SELDI-TOF for the selection of candidate serum biomarkers of vitamin E, selenium, and lycopene–supplementation. The same purified peptide-containing fraction was then fractionated by SDS/PAGE. The selected protein-containing band was in-gel digested and analyzed by LC-MS/MS for peptide identification. It was observed that combinations of micronutrients showed synergistic effects as preventative therapy for the progression of prostate cancer in transgenic mice model systems (Cervi et al., 2010).

At present, isoflavones are used as functional ingredients in a wide range of novel foods because there is considerable interest in their potential health benefits. Isoflavones are complex molecules with multiple biological activities, including prevention of cardiovascular diseases, neurodegenerative diseases, osteoporosis, cancer, obesity, or aging. Rowell, Carpenter & Lamartiniere demonstrated for the first time the usefulness of proteomics for the discovery of novel pathways that might be involved in cancer prevention by isoflavones (Rowell, Carpenter & Lamartiniere, 2005). For a better understanding of the pathways for the metabolism of isoflavones, a liver proteome was studied on rats treated with isoflavone rich extracts of red clover. For this pursose, total liver proteins were separated by 2-DE, and proteins which showed differences in their intensities were identified by MALDI-TOF-MS. A significant upregulation of 3-hydroxy-3-methylglutarly-CoAsynthase, and a down-regulation in peroxiredoxin 4 and 3-a-hydroxysteroid dehydrogenase were observed upon red clover treatment as compared to untreated controls, what support the potential of isoflavone rich red clover extract to modulate the lipid metabolism (Pakalapati et al., 2009).

Polyphenols, abundant in plant-derived foods particularly fruits, seeds, and leaves, and their beneficial effect for disease prevention have also been studied from a proteomic point of view. Proteomic results revealed the positive effects of red wine polyphenol compounds for stroke protection either as prevention or treatment of the different phases of the disease (Ritz et al., 2008).

The antiobesity effect of capsaicin, a major ingredient in hot pepper, has also been studied in rats (Kim et al., 2010, Joo et al., 2010). Through the comparative proteome analysis of white adipose tissue, those authors identified proteins involved in lipid metabolism, redox processes, and signal and energy transduction to provide important information about the mechanism of the antiobesity effects of capsaicin.

Chronic alcohol consumption has been studied from a proteomic point of view (Fogle et al., 2010). Cleavable isotope coded affinity tags (ICAT) technology in combination with SDS/PAGE for protein fractionation and MALDI-TOF-MS of the resulting labelled peptides from the SDS/PAGE gel slices, was used to identify differentially deregulated proteins in the myocardium of rats fed with a diet that contained ethanol. In general, myofibrillar, sarcoplasmic, membrane-associated, and mitochondrial proteins in cardiac muscle were reduced after chronic ethanol administration. The effect of a Platycodi Radix extract supplemented in diet of alcohol-exposed rats was studied by An et al. (An et al., 2009). Proteomic analysis revealed that 50 different proteins (involved with cytoskeleton regulation, signal transduction, cytokine, apoptosis, and reactive oxygen species metabolism) showed significant quantitative changes. After identification of these proteins, results suggested that the

antioxidant activity associated to Platycodi Radix intake might play a protective role on liver tissues of chronically alcoholic rats.

The piglet was used as an animal model to study the therapeutic effect of Zn on intestinal function in neonates (Wang et al., 2009b). A zinc oxide-supplemented diet showed the beneficial alteration of intestinal proteins related to the regulation of oxidative stress, redox state, cell proliferation, and apoptosis processes. The effect of maternal fatty acid nutrition on the protein expression in the neonatal offspring liver in rats has also been studied using conventional proteomic approach combining 2-DE and MALDI-TOF MS (Novak et al., 2009). The study on the up- and down-regulated proteins revealed that early fatty acid nutrition impacts hepatic metabolic pathways relevant to luconeogenesis, redox balance, and nitric oxide signaling.

The discovery of nutritional biomarkers offers great potential to understand the relationship between diet and health. A proteomic study of the intake of selenomethylselenocysteine (SeMSeCys), a chemoprotective form of selenium, was carried out by Mahn et al. (Mahn, Toledo & Ruz, 2009). Protein-expression patterns by 2-DE in blood plasma were studied in model rats treated with a different selenium dose (SeMSeCys or sodium selenate) and supplementation periods. Apolipoprotein E and transthyretin proteins were proposed as potential biomarkers of chemoprotective selenium intake.

Metabolic impact of flavonoid intake was studied with GC-MS. Sample preparation methods were optimized for metabolite extraction from several different biological matrices; i.e., urine, plasma, fecal samples, and *in-vitro* colonic fermentation

models. The sample preparation and GC-MS methods were used to study the metabolic impact in urine samples after intake of cellulose capsules with a polyphenol-rich mix of red wine and red grape juice extracts. Multivariate data analysis based on orthogonal projection-discriminant analysis (OPLS-DA) was applied to determine any differences between control and intervention groups to obtain the clear classification that can be seen in Figure 7 (Grun et al., 2008).

Great advances have been achieved with LC-MS- and CE-MS-based approaches for metabolic profiling/fingerprinting (Klaus 2010, Ramautar, Somsen & de Jong, 2009). Llorach-Asuncion et al. proposed the study of the metabolome modification before consumption of cocoa powder and during several periods after consumption with a combined partial least square discriminant analysis (PLS-DA) and two-way hierarchical clustering (two-wayHCA) to improve the analysis of the complex set of data obtained with HPLC-Q-TOF (Llorach-Asuncion et al., 2010).

III. FOODOMICS, MS-BASED METHODOLOGIES, AND SYSTEMS BIOLOGY.

MS-based 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, proteomics and metabolomics (plus transcriptomics) represents powerful analytical platforms developed for the analysis of proteins and metabolites (plus gene expression). 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) that give rise to the growth of a new discipline called Systems Biology (Hood et al., 2004). 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 (Panagiotou & Nielsen, 2009). 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, metabolite).

Although Systems Biology has been scarcely applied to Foodomics studies, its potential is underlined by its adoption by other disciplines. For instance, a Systems Biology approach has been applied to investigate carbohydrate metabolism in yeast (Weston & Hood, 2004). In a recent research, Kohanski et al. used the context likelihood of relatedness (CLR) algorithm (gene network analysis) in combination with gene expression microarrays and Gene Ontology-based enrichment analysis to construct and filter gene connectivity maps of bacteria under antibiotic treatment (Kohanski et al., 2008). The gene networks were further enriched with data derived from antibiotic growth high-throughput screening to provide insight into the pathway whereby the antibiotic under study triggers its bactericide action.

Recently, Systems Biology has been applied to understand the complexity of the processes in the intestinal tract (dos Santos, Muller & de Vos, 2010). This study is based on human adult microbiota characterization by deep metagenomic sequencing, identification of several hundreds of intestinal genomes at the sequence level, identification of the transcriptional response of the host and selected microbes in animal

model systems and in humans, determination of the transcriptional response of the host to different diets in humans, germ-free and gene knockout animals, together with different metabolomics and proteomics studies. Based on these data, an integrated, modular modelling framework that cross-links top-down and bottom-up approaches for the various levels of biological organization is proposed to understand intestinal function (dos Santos, Muller & de Vos, 2010).

D'Alessandro et al., have compiled and exhaustive list of 573 bovine milk proteins and elaborated the data using bioinformatic tools in order to retrieve relevant information about the functional role of bovine milk proteins (D'Alessandro, Zolla & Scaloni, 2011). An interactomics approach was applied for the first time in a food matrix that allowed the integrated study of the individual pathways, networks, and ontologies depicted. Bovine milk interactome is expected to be refined in the future using quantitative methods in protein interaction studies,

IV. FUTURE TRENDS IN FOODOMICS.

MS-based tools will have to overcome important limitations for optimal implementation in Foodomics in the non-distant future. In proteomics, MS as a stand-alone technique or combined with 2-DE, liquid chromatography, and capillary electrophoresis has become widespread. However, there is an evident need to develop 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. Separate from more-sophisticated sample treatments and separation techniques, MS will continue being essential for the systematic investigation in proteomics. In this sense, conventional mass spectrometers are replaced by 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 lower number of proteomic applications in Foodomics compared to the use of metabolomics-based approaches, it is expected that the application of these new instruments together with further technological innovations will help proteomic profiling to become a standard practice also in Foodomics. New applications of proteomics technologies are expected in the study of microbial flora in gut (Gilad et al., 2010) or on the use of functional proteomics in Foodomics (Schittmayer et al., 2009). As an example, although MS-based proteomics has proven to be a very useful tool for the identification, characterization, and detection of food allergens, still there are some issues that have not been successfully resolved, such as the development of MS-based methods for the simultaneous determination of multiple food allergens in food products and commodities.

Great advance is expected in metabolomics with the incorporation of new MS interfaces for which nearly no sample preparation is needed (Chen et al., 2006; Feng et al., 2008; Huang et al., 2007). Comprehensive multidimensional techniques, such as GCxGC or LCxLC, are also a revolutionary improvement in separation techniques that will be implemented in metabolomics studies in the near future. They might provide not only an enhanced resolution and a large increase in the peak number, but also an increase in selectivity and sensitivity in comparison with conventional separation techniques. As an example, comprehensive GCxGC coupled to TOF-MS is a promising tool for metabolic profiling (Pasikanti, Ho & Chan, 2008). Also, capillary electrokinetic techniques and their coupling to mass spectrometry (CE and CE-MS) are ideal tools for metabolomics, due to their minimal sample-preparation requirements, wide range of

applications, great efficiency and resolution, and low sample consumption. Although CE and CE-MS have not been widely used in Foodomics (Herrero et al. 2010), they have already been identified as a very promising tool for metabolomic studies (Garcia-Villalba et al., 2008; Levandi et al., 2008; Oh et al., 2010). Interesting examples on the use of CE-MS in Foodomics can be found in very recent research, such as the study of substantial equivalence of transgenic and conventional soybean from their peptidic profiles with a shot-gun approach (Simó et al., 2010).

The challenge in the combination of Foodomics and Systems Biology is not only at the technological level, where, as mentioned above, great improvements are being made and expected in the 'omics' technologies, but also on the bioinformatics side (data processing, clustering, dynamics, or integration of the various 'omics' levels) that will have to progress for Systems Biology to demonstrate all its potential in the new Foodomics discipline (Gehlenborg et al., 2010). In this regard, it is also interesting to mention that the traditional medical world has often noted that, although many of the omics tools and Foodomics approaches provide academically interesting research (Breikers et al., 2006; Fardet et al., 2007; Griffiths & Grant, 2006; Narasaka et al., 2006; Rezzi et al., 2007; Smolenski et al., 2007), they have not been translated to methods or approaches with medicinal impact and value because the data integration when dealing with such complex systems is not straightforward (Hirai et al., 2004; Schnackenberg et al., 2006). Thus, traditional medicine represents an important challenge for Systems Biology. A good example of the complexity that Systems Biology has to face in the Foodomics field is the study of the interplay of food, microbiota, and host related to intestinal functions that can be only understood from a systems perspective. The long-term goal is to understand how specific nutrients, diets,

and environmental conditions influence cell and organ function, and how they thereby impact on health and disease. This systems knowledge will be pivotal for the development of rational intervention strategies for the prevention of diseases such as diabetes, metabolic syndrome, obesity, and inflammatory bowel diseases.

In the future, Foodomics approaches can help to overcome the important limitations detected by several regulatory institutions, including the European Food Safety Authority (EFSA), related to the controversial demonstration about the health claims on different functional foods and food ingredients. Moreover, this approach can be extended to better prove (or not) the health claims that link health benefits to many other different compounds, most of them rejected by EFSA so far. In this regard, it has been mentioned that it is probably too early to conclude on the value of many substances for health. Thus, Foodomics could help to overcome the main limitations detected by EFSA to reject these proposals, namely: lack of information to identify the substance on which the claim is based; lack of evidence that the claimed effect is indeed beneficial to the maintenance or improvement of the functions of the body; lack of human studies with reliable measures of the claimed health benefit.

This knowledge can be better generated with multidisciplinary approaches that consider international consortia and working on Foodomics based on extensive populations. Foodomics can also be important in terms of public health by considering two different approaches: at short term, involves the clinical application to treat metabolic alterations such as diabetes, and at long term, more related to the public primary prevention-that means, to inhibit the development of disease before it occurs. It

is clear that MS-based strategies will play a definitive role to solve these huge challenges in the new Foodomics field.

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FIGURE LEGENDS

Figure 1. Foodomics: covered areas, tools, and goals.

Figure 2. Scheme of the different strategies that can be followed to carry out a proteomic study.

Figure 3. Three-dimensional and contour plot obtained after the analysis with GCxGC-TOF-MS of a standard solution that contains 360 pesticides (reproduced from Van der Lee et al., 2008).

Figure 4. MALDI-TOF-MS spectral profiles of *Enterobacter spp., R. planticola*, *Klebsella spp*., and *Providencia spp* with species-specific peaks indicated with an asterisk, genus-specific peaks with a circle, and further characteristic peaks with a triangle (reproduced from Bohme et al., 2010).

Figure 5. Transformation process of protein ion-mass spectral barcodes from a MALDI-TOF-MS spectrum of proteins extracted from a honey sample (left): A) MALDI-TOF-MS raw mass spectrum, B) graphic output of the identification results displayed within the graphic view, C) the peaks transformed into barcodes; and MALDI-TOF protein mass spectral barcodes and selected enlargements (right) of 16 different honeys of known origin (reproduced from Wang et al., 2009a).

Figure 6. CE-TOF-MS base-peak electrophoregram of the digested protein extract from conventional and transgenic soybean (Redrawn from Simó et al., 2010).

Figure 7. Profile of phenolic acids in urine after intake of grape juice/wine extract. (A) GC–MS profiles of ethyl acetate extracts of human urine of placebo (top chromatogram) and intervention (bottom chromatogram). (B) OPLS analysis of the GC–MS profiles of urine to show the different metabolic impact of intervention vs. placebo intake. (C) OPLS coefficients plots to indicate the metabolites that increased after intervention (Redrawn from Grün et al., 2008).

Table 1. Some representative Foodomics applications for the simultaneous analysis with MS-based methodologies of more than 30 pesticides and/or antimicrobials in foods.

Compounds determined	Food	MS based tool	Sensitivity		Ref.
			LOD	LOQ	
31 antimicrobials (including β-lactams, lincosamides, macrolides, quinolones, sulfonamides, tetracyclines, nitroimidazoles and trimethoprim)	Meat (bovine and pork)	LC-ESI-MS/MS (QqQ in SRM mode)	3-10 μg kg^{-1}	15-50 μg kg^{-1}	Carretero, Blasco & Pico, 2008
44 pesticides	Raw bovine milk	LC-ESI-MS/MS (QqQ in SRM mode)	0.05-3 ng g^{-1}	0.2-10.1 ng g^{-1}	Dagnac et al., 2009
Antibiotics (penicillins, cephalosporins, sulfonamides, macrolides, lincosamides, aminoglycosides, tetracyclines, and quinolones)	Milk	LC-ESI-MS/MS (QqQ in MRM mode)	4-100 μg kg^{-1}		Gaugain-Juhel et al., 2009
106 pesticides and contaminants	Cereal products	GCxGC-TOF-MS		1-20 μg kg^{-1}	Van der Lee et al., 2008
38 anthelmintic (including benzimidazoles, macrocyclic lactones, and flukicides)	Milk, liver	LC-ESI-MS/MS (QqQ in SRM mode)		5-10 μg kg^{-1}	Kinsella et al., 2009
47 antibiotics (tetracyclines, quinolones, macrolides, sulfonamides, diamino-pyrimidine derivatives and lincosamides)	Milk	LC-ESI-MS/MS (QqQ in SRM mode)	[a]6.8-243 μg kg^{-1}	[b]8.1-325 μg kg^{-1}	Bohm, Stachel & Gowik, 2009
148 pesticides	Berry fruits	LC-ESI-MS/MS (QqQ in MRM mode)		> 5 μg kg^{-1}	Wang, Leung & Chow, 2010
46 pesicides	Wines	LC-ESI-MS/MS (QqQ in SRM mode)	0.3-3 μg L^{-1}	1-10 μg L^{-1}	Economou et al., 2009
191 pesticides	Fruits	LC-ESI-MS/MS (QqQ in MRM mode)	0.5-5 ppb		Wong et al., 2010a
140 pesticides	Cereals	GC-MS/MS (QqQ in MRM mode)		0.01 mg kg^{-1}	Walorczyk, 2008
205 pesticides	Vegetables, fruits and beans	LVI-GC-MS (SIM mode)	0.15-200 μg kg^{-1}	0.5-600 μg kg^{-1}	Xu et al., 2009
130 pesticides (including insecticides, herbicides, fungicides and acaricides)	Orange, nectarine and spinach	GC-MS/MS (QqQ in MRM mode)	0.1-50 μg kg^{-1}	< 0.01 mg kg^{-1}	Cervera et al., 2001

33 multiclass pesticides	Fruit-based soft drinks	UPLC-ESI-TOF-MS		0.02-2 μg L^{-1}	Gilbert-Lopez et al., 2010
101 pesticides (including triazines, organophosphorous, carbamates, phenylureas, neonicotinoids)	Vegetables	LC-ESI-TOF-MS	0.04-150 μg kg^{-1}		Ferrer & Thurman, 2007
140 pesticides	Cucumber and orange	GC-MS/MS (QqQ in MRM mode)		10 μg kg^{-1}	Fernandez-Morenoet al., 2008
167 pesticides (organohalogen, organophosphorus ad pyrethroid)	Vegetables and fruits	GC-MS/MS (QqQ in MRM mode)		3.4 μg kg^{-1}	Wong et al., 2010
102 pesticides	Tea	GC-MS (SIM mode)		0.012-1.5 μg ml^{-1}	Huang et al., 2007
100 pesticides	Strawberry	UPLC-TOF-MS	0.02 mg kg^{-1}		Taylor et al., 2008
42 pesticides	Tea	LC-MS/MS (QqQ in MRM mode)		4-45 μg kg^{-1}	Kankar, Mandal & Bhattachatyya, 2010
98 pesticides (organophosphorous and carbamates) and related products	edible oil, meat, egg, cheese, chocolate, coffee, rice, tree nuts, citric fruits, vegetables	LC-MS/MS (QqQ in MRM mode)		10 μg kg^{-1}	Chung & Chan, 2010
82 pesticides	Pomegranate, apple, orange	LC-MS/MS (QqQ in MRM mode)		2.5-5.0 μg kg^{-1}	Banerjee et al., 2008

[a] CCα, decision limit; [b] CCβ, detection capability

Table 2. Some representative Foodomics applications in the field of Nutrigenomics that used MS-based proteomics.

Bioactive compound (supplemented-diet)	Studied model	Issue	MS based tool	Ref.
Vitamin E, selenium and lycopene	12T-10 transgenic mice plasma	Prostate cancer	SDS/PAGE, LC-IT-MS	Cervi et al., 2010
Genistein, biochanin A, formononetin, glycetin (ISOFLAVONES) from red clover	Rat liver	Metabolism influence	2-DE, MALDI-TOF-MS	Pakalapati et al., 2009
Red wine polyphenols	Rat brain	Cerebral stroke prevention	2-DE, MALDI-TOF-MS	Ritz et al., 2008
Capsaicin	Rat skeletal muscle cells Rat white adipose tissue	Antiobesity effect	2-DE, MALDI-TOF-MS	Kim et al., 2010; Joo et al., 2010
Ethanol	Rat cardiac Muscle	Chronic alcohol consumption	ICAT, nLC, MALDI-TOF/TOF-MS	Fogle et al., 2010
Platycodi Radix extract	Rat liver	Alcoholic liver disease	2-DE, MALDI-TOF-MS	An et al., 2009
Zinc	Piglet intestine	Intestinal function	2-DE, MALDI-TOF-MS	Wang et al., 2009b
Poly unsaturated fatty acids	Neonatal rat liver	Hepatic metabolic pathways study	2-DE, MALDI-TOF/TOF-MS	Novak et al., 2009
Selenomethylselenocysteine	Rat plasma	Chemoprotectice selenium intake biomarkers	2-DE, MALDI-TOF-MS	Mahn, Toledo & Ruz, 2009