Novel Mass Spectrometry-Based Applications of the 'Omic' Sciences in Food Technology and Biotechnology

Gianluca Picariello[1], Gianfranco Mamone[1], Francesco Addeo[2] and Pasquale Ferranti[1,2]*

[1]Institute of Food Science and Technology (ISA) – CNR, Via Roma 52 A/C, IT-83100 Avellino, Italy

[2]Department of Food Science, University of Naples 'Federico II', Parco Gussone, IT-80055 Portici, Italy

Summary

The revolution of 'omic' sciences has introduced integrated high-throughput approaches to address the understanding of the biochemical systems and of their dynamic evolution. In the field of food research, 'omics' are depicting a comprehensive view which largely overcomes the merely descriptive approaches of the early proteomic and metabolomic era. Thus, the recently born 'foodomics' is to be intended as a global perspective of knowledge about foods, which covers the assessment of their composition, the effects of (bio)technological processes for their production, their modifications over time and the impact that food consumption has on human health. Food proteomics and metabolomics, along with their derived 'omic' branches such as peptidomics, lipidomics and glycomics, are still evolving technologies capable of tackling the nature and the transformations of foods. In the development of the advanced 'omic' platforms, because of their potential to profile complex mixtures of biomolecules, mass spectrometry techniques have assumed an unquestionable role. Because proteins are central molecules in all biological systems, proteomic platforms are pivotal among the 'foodomic' tools, as the proteomes and related peptidomes provide biomolecular subsets mostly informative about the history of a food product. Similarly, food interactomics and metabonomics aim to study the dynamics that occur in foodstuff. The ultimate aim of foodomics is the production of high-quality and safe food products for improving human health and well-being. In this review we critically present the recent research outcomes in the field of food sciences that have been achieved through the contribution of the 'omic' methodologies relying on mass spectrometry.

Key words: proteomics, peptidomics, metabolomics, interactomics, mass spectrometry, food proteins and peptides, food quality, food safety, food technology

Introduction

The comprehensive characterization of food composition and the study of the modifications induced by the technological processes are basic issues of food science. Strictly related to food science is nutrition, intended as the discipline which addresses the knowledge of how food is processed after ingestion and its effect on human health.

Foods are, in general, mixtures of very different chemical compounds and they reflect the complexity of the animal or vegetable organism from which they derive. Just like any other biological system, the recently born 'omic' sciences, intended as the systematic definition of subsets of specific biomolecules, are finding applications also to foods. Thus, for instance, food proteomics includes the complete cartography of the proteins, while metabo-

*Corresponding author; Phone: ++39 81 253 9359; Fax: ++39 81 776 2580; E-mail: ferranti@unina.it

lomics studies the complement of small molecules (low molecular mass arbitrarily assigned below 1–2 kDa) of a foodstuff. Phosphoproteomics, glycomics, lipidomics concern specific classes of constituents of the systems under analysis. In particular, in consideration of the relevance of biological systems of lipids and sugars, lipidomics and glycomics are emerging as self-standing 'omics' from the wider field of metabolomics.

Considering that the different 'omic' sciences are all inter-related (Fig. 1), within the course of biological and technological production of a food matrix as well as in the process of food modification, the concept of 'interactomics', intended as the study of the interactions among biomolecules, also holds in food science. While nutrigenomics, *i.e.* the discipline that studies the effects of food constituents on gene expression, is an emerging science (*1*), which, however, goes beyond the subject of this review, food genomics is most commonly restricted to the application of technologies for the characterization of DNA in order to establish the origin of the raw materials. Time is also ready for launching the systematic genome-wide analysis of diet-induced alterations of the chromatin structure without changes in the nucleotide sequence (epigenomics). Epigenetic changes affect gene expression and, hence, have significant consequences for human health. Non-secondary application of food genomics and transcriptomics, also combined with proteomics and metabolomics (*2*), entails the design and the study of genetically modified organisms (GMO), the related assessment of substantial equivalence and the evaluation of the risk of

unintended effects. These issues have been extensively covered in very recent reviews (*3,4*) and will not be further discussed here.

In the perspective of the global knowledge of foods, the concept of foodomics, more than a simple term covering the panel of the 'omic' sciences, also includes the nutritional aspects (*4*). The core of the 'omic' sciences, and hence of foodomics, are the 'omic' technologies that rely on the currently well established analytical platforms. In particular, mass spectrometry (MS) techniques are crucial and are assuming an even increasing role for the analysis of the proteomes, peptidomes and metabolomes.
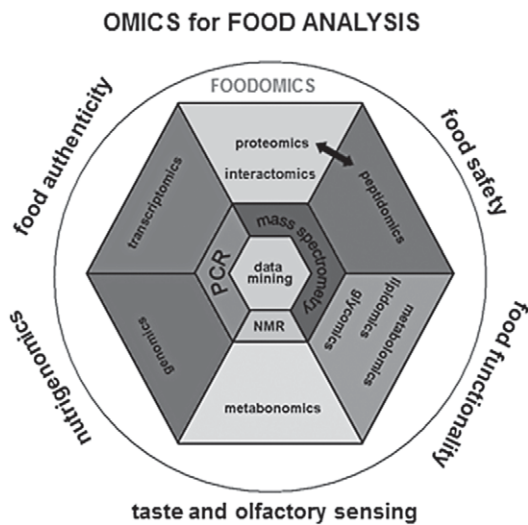
The huge harvest of data generated by the 'omic' technologies has required the development of bioinformatic strategies of storage and organization to definitely convert raw data into useful information. The parallel and interconnected implementation of 'omic' and bioinformatic tools is on its full course, so that further methodological progress is to be envisaged even in the near future.

The astonishing advances in food science have prompted the development of totally new (bio)technological processes for food preparations, and the growing knowledge about food functionality, bioactivity and toxicity has been raising novel and urgent questions. Many excellent recent reviews cover selected topics among the aspects that concur with foodomics. Thus, far from the intent to provide an exhaustive survey of literature concerning the application of 'omic' tools to food analysis, this review critically presents newest exemplificative achievements of 'omic' sciences generated by the synergistic efforts of researchers in the fields of analytical chemistry, in particular mass spectrometry, with those working in food technology and biotechnology. These developments are driven by the need to provide solutions for the requirements of producers, food industry, regulatory agency, and consumers in order to improve food quality, functionality and storability, and to optimize the effects of technological processes on food components with the ultimate objective of improving human health and wellness.

Fig. 1. The integration of the 'omic' sciences and technologies in food analysis (foodomics), a global perspective of the knowledge of foods. Ultimate aims of foodomics are the assessment of food safety and food authenticity, the study of the mechanisms of taste and olfactory sensing. These aspects, combined with the determination of the impact of foods on human health will fuel the design and development of food products with ameliorated sensory properties, tolerability and with controlled functional effects. The specific effect of food components on gene expression is the basis of the nutrigenomics that is heralding the era of the individualized nutrition. The primary (but not exclusive) technological platforms which 'omic' sciences rely on are indicated. The whole body of the 'omics' converges in the creation of bioinformatic tools for data storage and conversion (data mining)

Mass Spectrometry Techniques in 'Omic' Sciences

Mass spectrometry (MS) in combination with separation methods and bioinformatic tools is the key analytical technique on which the emerging 'omic' technologies such as proteomics and metabolomics are based. In particular, in the field of food sciences, MS-based techniques have rapidly evolved for the determination of quality, authenticity, functionality and safety issues. Numerous reviews of MS tools have been published (*5–7*). In the following sections, we briefly review the main MS technologies applied to food analysis.

Several modes of analysis are available in MS which differ markedly by the ionization source. In proteomic research electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are the two techniques most commonly used for MS (*8,9*). With these tools at hand, it has become feasible to identify proteins by comparing sets of peptide masses (peptide mass fin-

gerprints, PMFs) or by fragmenting peptides selectively isolated in the mass spectrometer in order to read out the (partial) amino acid sequence (MS/MS) (*10*). The still ongoing development of multi-stage MS instruments with enhanced mass accuracy (up to low ppm) and sensitivity (low attomole level) has rendered MS an extremely powerful tool for protein identification by peptide sequencing (*11*).

All MS-based proteomic approaches consist of three distinct stages: (*i*) isolation and (pre)fractionation of protein sample, (*ii*) qualitative and quantitative analysis by MS or MS/MS, and (*iii*) assignment of MS or MS/MS spectra to peptides or proteins through database searching (*7*). This workflow can be performed through two fundamental analytical strategies: the bottom-up or the top-down approaches. Both methodologies differ in the separation requirements and the type of MS instrumentation. The original bottom-up strategy is accomplished by the combination of two-dimensional gel electrophoresis (2-DE) followed by MS and database search. After image analysis of the 2-DE gels, the target proteins are submitted to an in-gel digestion (*i.e.* trypsin) and identification by MALDI-TOF-MS (peptide mass fingerprinting, PMF) or liquid chromatography (LC)-tandem mass spectrometry (MS/MS) (*10,11*). The 2-DE approach allows to resolve very complex proteomes. However, it is time consuming, suffers from low sensitivity, and depends strongly on staining and visualization mode adopted (*12*).

A gel-free alternative strategy to 2-DE is an advanced bottom-up approach named shotgun or multi-dimensional protein identification technology (MudPIT) (*13*). This methodology consists in the previous digestion of the whole protein mixture and multi-dimensional (*i.e.* SCX-RP, alkaline RP-HPLC) chromatographic separation of the peptides, followed by on-line LC-MS/MS analysis (*14,15*). In spite of the higher sample complexity due to the huge number of peptide species in the protein mixture hydrolyzate, peptides are in general separated and analyzed by MS much better than proteins. Shotgun approach requires the use of high resolving power LC-MS/MS instruments such as quadrupole time-of-flight (Q/TOF), TOF/TOF, and the most recent hybrid linear ion trap (LTQ) Orbitrap (*5*). ESI source is the interface of choice to ionize peptides eluted from LC due to the possibility of continuous sample infusion into the ESI source (*16*). Proteomic studies have also focused on the use of MALDI interface for LC separations in an automated, but off-line mode (LC-MALDI-TOF/TOF) (*17*). However, one of the major drawbacks of the gel-free approach is that the information on the intact protein is lost, which implicates that information on protein break-down (*i.e.* by proteases) is usually not available unless the complete protein sequence is covered. This limitation can be important in the case of fermented foods (yogurt, cheese, sausage), where proteins undergo extensive proteolysis.

An emerging MS strategy alternative to bottom-up is the top-down proteomics. After ionization proteins are fragmented in the gas phase, generating amino acid sequence information without previous proteolytic digestion or other chemical processing (*18,19*). The main advantages with respect to the bottom-up approach are the higher sequence protein coverage and the identification

of post-translational modifications (PTMs). Because of their high resolution, Fourier transform ion cyclotron resonance (FTICR) and Orbitrap instruments, especially when coupled to electron transfer dissociation (ETD), are the most effective instruments for top-down proteomics (*20,21*).

Similar to proteomics, the metabolomics workflow implies a combined use of advanced analytical techniques (mainly 'omic' tools), and bioinformatic methods to recover relevant metabolic information (*22*). Analytical methodologies are based on traditional gas chromatography (GC) and LC techniques coupled with MS (GC-MS, LC-MS). GC-MS analysis has been extensively used in metabolomics because of its high separation efficiency and easy interfacing of GC with MS. It can be used to analyze a wide range of volatile and chemically derivatized semi-volatile compounds. However, sensitive and specific LC-MS methods have been extensively used instead of GC-MS because derivatization of polar/nonvolatile metabolites for GC-MS analysis is both time-consuming and often requires more material if compared to LC-MS (*23*). A recent introduction into the field of metabonomics is ultra-performance liquid chromatography (UPLC) system, with narrow columns either monolithic or packed with small diameter particles, coupled to MS/MS instruments. Compared with HPLC-MS system, UPLC-MS system has very short run time, higher sensitivity and resolution, and signal-to-noise (*S/N*) ratio that could be increased by fivefold (*24*).

Food Proteomics

Assessment of raw material quality

The application of the 'omic' technologies in food science is aimed to define the entire and detailed (bio)chemical composition of food and its modification throughout the production process to correctly evaluate its nutritional, toxicological and functional properties.

The proteome reflects very closely the (bio)chemical processes a system undergoes. The proteins of food are at the same time a passive source of amino acids but also an active food component giving food its textural, functional and sensory characteristics. Proteins also play an active crucial role in inducing food modifications during production, maturation and storage, by catalysis of processes such as oxidation, proteolysis or lipolysis. Therefore, proteins are largely responsible for the overall characteristics of most food preparations. Because of the potential to generate a systematic view of the protein composition and of the biological as well as chemical interactions among proteins, the application of proteome analysis in food science is steadily growing. The aim of these studies is to define a complete database of the protein composition of a given food product, and, more importantly, to relate structural to functional characteristics of the food itself.

Nutritional, rheological and sensory properties of milk-, cereal-, and meat-derived products depend on the composition of the protein fraction. This is in turn determined by the combination of genetic factors with those introduced by the technological process.

The proteins present in the raw materials are essential in determining the end-product quality. For instance, it is well known that protein composition determines curding aptitude and cheese yield from milk. The influence of milk protein qualitative and quantitative polymorphism on the cheese-making process was recognized in the early 1970s (*25*), but traditionally polymorphism analysis has been carried out with merely descriptive approaches (electrophoresis, chromatography), whereas the structural basis of the phenomenon remained unidentified. One of the first cases (at the time the concept of 'proteomics' itself was still at the beginning) where MS showed its full potential in providing a molecular key to understanding the technological behaviour was the characterization of the genetic variants of α_{s1}-casein in ovine milk (*26*). LC-ESI-MS was applied to assign the structural difference between the common α_{s1}-casein variant C and the less spread variant D. In a single experiment both amino acid sequence and PTMs were assigned, showing that a simple Ser/Asn replacement cancelled the phosphorylation cluster responsible for the efficient milk curding process; therefore, as a consequence of the structural variation, milk containing variant D showed the worst aptitude to coagulate and poor cheese yield. Thus, the structural characterization of a protein variant allowed to link the occurrence of non-allelic deletion and consequent alteration in casein micelle characteristics with cheese-making aptitude (*27*). This example shows how the characterization of alleles at the protein level by MS can provide the necessary information for developing typing procedures aimed to increase the efficiency of selection and breeding programs, which ultimately contribute to the improvement of the cheese-making quality of milk. Since then, the MS ability to provide structural protein characterization by analysis of peptide mixtures generated by enzymatic or chemical cleavage (Fig. 2), has allowed to define the entire panel of milk proteins in ruminant species including sheep (*28,29*), goat (*30*), buffalo (*31*), as well as in human (*32*), horse (*33*) and donkey (*34,35*). Later on, the evolution of proteomic techniques allowed to one-step trace the entire descriptive milk proteome (caseome) (*36*) and phosphoproteome (*36,37*), thereby replacing the previous single-protein targeted approaches.

Caprine milk is a further example highlighting the role of quantitative protein polymorphism in food characterization (*25*). There are marked technological and sensory differences between goat cheese produced with milk containing 'strong' alleles (*i.e.* those containing high α_{s1}-casein levels) and that made with milk containing 'weak' alleles. These differences include hardness/softness and aptitude to ripening. The complexity of this protein system required the development of a peptide-centric approach based either on MALDI-TOF-MS or LC-ESI-MS/MS, in which signature peptides were most conveniently targeted as analytical surrogates of the parent proteins (*38*). In this manner, internally deleted goat α_{s1}-casein has been detected and quantified in bulk milk (*39*). Because of the strong influence of the casein variants on the quality of milk and on the clotting aptitude, the strategy provides an analytical means to help farmers and milk producers to decide in advance the destination of milk for drinking or for cheese making.

However, the most remarkable field of application is probably that of wheat flour-derived products, whose optimal technological characteristics are ultimately determined by the gluten proteins (gliadins and glutenins). The abundance and the molecular characteristics of gluten proteins determine the dough-making aptitude of flour (*40*). As in the case of milk, the conventional classification based on electrophoretic mobility has been made obsolete by DNA analysis: the number of genes encoding these proteins is extremely high (greater than 100 copies per haploid genome) (*41*). HPLC coupled on-line with ESI-MS or off-line with MALDI-TOF-MS represents an efficient tool for obtaining complementary data for the characterization of the gluten complex (*42–44*).

One of the first applications was the LC-MS analysis of gliadin profiles from durum wheat to differentiate the cultivars in terms of the nature and abundance of the protein components (*42*). Although different cultivars showed similar chromatographic profiles, the determination of M_r protein components with similar retention times are often different among the varieties, due to the occurrence of amino acid substitutions, insertions, or deletions in the gliadin sequences. A certain degree of structural heterogeneity also characterizes high molecu-
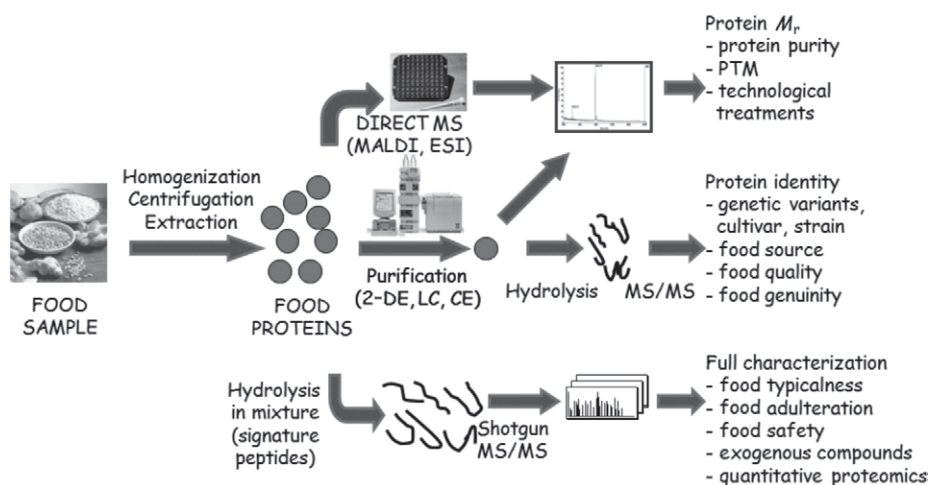

Fig. 2. Proteomic approaches to food analysis

lar weight glutenin subunits (HMW-GSs). These storage proteins are minor components of gluten fraction in terms of quantity, but they are key factors in the process of bread making. In fact, HMW-GSs are the major determinants of gluten elasticity, promoting the formation of larger glutenin polymers (40).

When the bottom-up approach based on 2-DE and MS was applied to the investigation of gluten proteins, it became apparent that the molecular characterization of gluten has to face many challenges. Gliadin and glutenin proteins are encoded by a high number of genes with similar structural characteristic, resulting in a complex protein mixture, which in addition produces a very limited number of tryptic peptides suitable for MS analysis. To overcome the latter limitation, alternative endoproteases (*i.e.* chymotrypsin) were used for digestion of proteins excised from 2-DE gel (45–47). By coupling database searching with *de novo* sequencing of the MS/MS data of the chymotryptic peptides in each spot, sequences common to all isoforms belonging to the given gliadin subfractions could be detected, as well as the sequences differentiating various isoforms of the same proteins. The combination of 2-DE, MALDI-TOF-MS and LC-MS/MS allowed the almost complete coverage of glutenin sequence (48). This approach has also clarified the complete absence of PTMs, suggested in previous experimental and theoretical studies (49,50). In a recent work, this analytical approach allowed the identification of a truncated form of the subunit By8 (51). The presence of this truncated form was explained with either endogenous proteolytic cleavage of the full-length protein or by gene deletion events occurring in the HMW-By8 allele. In addition, by this approach the avenin-like protein type-B has been detected as a novel non-gluten component, associated with gluten (48,51).

Authentication and traceability

Protein mapping by combining high resolution separation techniques with MS has also provided important outcomes in the field of food authentication, in accordance with the comprehensive system of authentication and traceability of food and feed recently established by the European Food Safety Authority (EFSA) in order to ensure food safety. The issue of food authentication, particularly urgent in the case of Protected Denomination of Origin (PDO) products, also requires efficient traceability systems, to ensure the correct application of the procedures throughout the productive processes and during distribution. The analytical response which includes sensitivity, specificity and speed in MS-based techniques represents the most accurate strategy to face the challenging tasks in this field. Moreover, proteins encrypt highly distinctive information about the identity and the history of a specific product. Thus, it is not surprising that targeted MS analysis of both proteins and proteolytic peptides has been largely developed for food authentication purposes.

The potential of MS to assess the quality of milk--based products has been reviewed (52). Several MS--based procedures have been developed to authenticate 'raw materials' used in manufacturing of dairy products. The adulteration of milk of higher commercial value with cheaper bovine milk is quite frequent. Taking advantage

of the species-specific amino acid substitutions along the homologous protein chains which affect molecular mass of both caseins and whey proteins, it is possible to define fingerprinting profiles of milk from different species either by electrospray or MALDI-TOF-MS, as shown for detection of bovine caseins in water buffalo mozzarella cheese (53,54). The LC-ESI-MS analysis of casein-derived tryptic peptides represents an even more powerful approach to the 'speciation' of milk, allowing to determine in a single experiment the content of milk from four ruminant species in mixtures with the aim of detecting foreign milk in cheese (55). In the assessment of meat and fish genuineness and in foodborne bacterial identification, MS-based techniques have the capability to substitute or complement other available strategies, such as the multiplex PCR assay (56).

Impact of technological processes

A food product is in general the result of a series of technological steps which are combinations of physical and chemical processes: thermal treatments, spray drying, cooking, extrusion, gel or dough formation, chemical or enzymatic hydrolysis, cross-linking, oxidation, just to mention a few, all of which induce deep and different structural changes in the food constituents. For this reason, while in raw food materials the characterization of protein constituents, still in a relatively 'native' state, can be considered quite standardized at present, proteomics of processed foods remains a challenging task and requires properly designed approaches. The main limiting factors to proteomic analysis of processed foods are the increased protein complexity (for instance, production of oxidized protein families or of mixtures of hydrolytic fragments), and the interaction of proteins with other proteins or with other molecules within the food matrix (for instance in the dough network formation or in the case of condensation products between carbohydrates and proteins in the early stages of the Maillard reaction) (56). However, these reactions are not simply an analytical problem but, on the other side, may also be precious markers of the type of process and of the modifications of food quality. For this reason, in the last years procedures of protein chemistry, sometimes refined from classical biochemistry protocols, have been developed or adapted to obtain efficient protein extraction and characterization from processed foods. As a first example, MALDI-TOF--MS has been used to study the alteration of gliadins during the baking process (57,58), and FTICR-MS demonstrated that the formation of tyrosine cross-links (other than the conventional disulphide bonds) during dough making also contribute to gluten development and to bread-making quality (59). The technological processes required for industrial food preparations may induce chemical modifications in gluten proteins potentially relevant for the nutritional and toxicological characteristics of the products. These modifications include deamidation of glutamine residues (60,61), formation of unnatural amino acids (such as lysinoalanine or lanthionine) and non-enzymatic glycosylation caused by heat treatments (62). In these cases, several markers of heat treatments have been identified and used to set up procedures to monitor either the correctness of the treatment on food matrices (milk, soy, juices, jams) or to detect the fraudu-

lent addition of prohibited ingredients in PDO products. For instance, the unnatural amino acid lysinoalanine has also been proposed as a marker to demonstrate either excessive heat treatments or the addition of heated milk or milk powders to fresh milk (*63*).

Heat treatment is applied not only to ensure microbiological food safety, but also to inactivate anti-nutritional components such as lectin phytohaemagglutinin (PHA) present in raw legumes. The normal range of lectin levels in bean (*Phaseolus vulgaris*) is 1–10 g/kg, which also implies a risk for health in processed food (*64*). The most common method of destruction is heat treatment in boiling water or by extrusion. Inaccurate heat treatments of beans have caused severe poisoning outbreaks in restaurants or refectories (*65*). Furthermore, commercial protein concentrates of common beans, the so-called 'starch-blockers', are more frequently used as dietary supplements to control body overweight in obesity. The development of specific and sensitive methods needs to rely on the lectin structural and functional definition, which can now be achieved by integrated proteomic and glycomic methodologies, able to define the structural change (proteolysis, oxidation, sugar changes), which can occur in raw and in industrially treated products. In this way, they constitute the basis to allow for either the structural or quantitative analysis of PHAs in the samples of different origin (*66*).

Proteomics in food preservation and safety

The 'omic' technologies can also help scientists to get better understanding of the life cycles of bacteria. Defining the mode of action of food-borne bacteria and the mechanisms that confer 'stress resistance' should enable more rational design of food preservation techniques, for instance using active antimicrobial compounds from natural sources (*67*). In addition, this information can also be used to pinpoint the areas of food chain that are most susceptible to microbial contamination.

In this respect, the analysis of pathogenic microorganisms is of special importance, as the risks associated with their contamination are not limited to their living presence and capacity of infectivity, but they can generally release protein/peptide toxins able to survive for a long time even in foods after bacterial cell contamination has been removed, as it happens for many of the microbes that cause food-related diseases (*Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Clostridium botulinum*, various *Salmonella* species). These pathogens release protein virulence factors, which have an essential role in the colonization of the host cells, into extracellular medium, and thus reflect the degree of bacterial pathogenicity. These toxins, being heat-stable and resistant to proteases, are a risk for the consumer's health. For the exploration of virulence factors expressed in the secreted proteome fraction, in a very recent study different *S. aureus* strains have been analyzed using gel-based bottom-up proteomic approach (*68*). A still more complex situation is expected for the analysis of food mostly constituted of a highly complex matrix of proteins, lipids, carbohydrates and many other molecular species that interfere with the detection of predictable toxin amounts (in the order of ppb). For this reason, the combination of MS methodologies with advanced immunochemical, chroma-

tographic and electrophoretic isolation procedures has to be applied. One such study has been carried out to define the toxin contamination levels of ripened PDO Italian cheese (*69*).

Food Peptidomics

Peptides dynamically change in food as the result of proteolysis, modifications induced by technological treatments and interactions with other components. They decisively affect both biological as well as functional properties of food products. Thus, food peptidomics, intended as the global characterization of the peptidome of a given food product, embraces a wide research area that aims to establish the origin of the peptidome, its evolution, the impact on the sensorial properties, and its beneficial or adverse effects on human health. Food peptidomics also encompasses the development of analytical strategies to study the food peptidome.

Generally speaking, with special concern for the MS-based techniques, peptides are by far better analytical targets than the parent proteins. These aspects underlie the so-called 'peptide-centric' approach to proteomics, which is considered to be the most powerful.

Peptides trace the technological processes that a food has undergone. Therefore, peptides are much more than just convenient analytical substitutes of parent proteins in many of the proteomic workflows; peptidome indeed provides uncomparable information about a given food product.

The change in peptide composition of cheese during ripening is a striking example. The peptide profile indicates the origin and the evolution of the protein fraction. A large use of MS techniques was made in the past to trace the proteolytic events that occur in the ripening of Parmigiano-Reggiano (*70,71*), Emmenthal (*72*) or Cheddar cheese (*73*). Peptidome profile also indicates the origin, authenticity and typicity of PDO cheese. Proteolysis as a function of the rennet typology has been assessed in Fiore Sardo ovine PDO cheese (*74*).

The MS methods, which have recently been reviewed (*75*), are the methods of choice for assessing proteolysis of dairy products. For instance, the pathways of extensive casein breakdown in human milk have been delineated by LC-ESI-MS (*76*). Peptide affecting taste and other sensorial traits can be identified by MS (*77,78*). A peptidomic approach based on capillary electrophoresis-MS allowed the identification of specific β-casein fragments, referred to as γ_4-casein, in buffalo milk arising from plasmin hydrolysis (*79*).

Although at a minor extent, proteolysis also occurs in frozen foods. This is the case of the γ-caseins produced even during the cold storage of buffalo curd (*80*). Interestingly, peptidome changes have also been observed in ice-stored fish, in addition to the expected changes due to cooking (*81*). The proteolytic peptides specifically generated are molecular targets to distinguish fresh from cold-stored materials, and therefore to assess the fraudulent use of unallowed refrigerated or frozen ingredients.

The biochemical changes that occur during the processing of dry-cured ham have been studied with peptidomic techniques relying on MS approaches. The intense

action of endogenous proteolytic enzymes that are activated in the early *post-mortem* phase provokes the extensive degradation of muscle proteins. The recent identification of a large number of small peptides released from enzymes belonging to the glycolytic pathway has confirmed that muscle sarcoplasmic proteins are relevant substrates for proteolysis (*82*). Generally, in real foods, as in this case, the identification of the released peptides is made difficult by the very broad or unknown cleavage specificity of the acting enzymes. Nevertheless, the multistage MS-based peptide sequencing can furnish information about the activity of early (calpains) as well as the medium- and long-term acting (cathepsins) endogenous proteases. The identification of oligopeptides characterized by the consecutive loss of terminal amino acids has demonstrated an additional intense secondary proteolysis in dry-cured ham, which is due to both amino- and carboxypeptidases (*83*).

The MS-based identification of large- and small-size fragments of myosin (heavy and light chains) and actin has demonstrated that muscle myofibrillar proteins also undergo extensive proteolysis in the maturation of dry--cured ham (*84–86*).

The proteolytic machinery of lactic acid bacteria concurs with a more extensive proteolysis of fermented sausages (*87*). However, few peptidomic investigations have been published related to these products. The peptidomic definition of proteolytic patterns could be helpful in assessing or predicting the suitability of specific strains of lactic acid bacteria as starter cultures for dry fermented sausages. The MS identification of species-specific peptide biomarkers has also been proposed as a tool for determining the authenticity of meat (*88*).

From a nutritional standpoint, rather than simply protein degradation products, peptides have proven to be important bioactive food components. They are released by hydrolytic events that occur in processing or formed during gastrointestinal digestion. Food bioactive peptides act as hormone-like species explicating opioid, anti-hypertensive, antimicrobial, immunomodulatory and other effects *in vivo*. MS is currently the elective technique to profile food hydrolytic peptides and for the targeted characterization of bioactive peptides. Many fragments released by proteolysis of meat and milk proteins have been claimed as biologically active peptides (*89*). The persistence and bioavailability of the bioactive peptides *in vivo* are still under debate. To this purpose, it has recently been demonstrated, by means of HPLC coupled to off-line MALDI-TOF/TOF-MS/MS, that some casein fragments of yogurt preparations supposed to be health--promoting are indeed degraded by bacterial cell-envelope proteases and are therefore no longer available (*90*). The source, the identification and determination of physiological actions of food-derived bioactive peptides have been exhaustively revised elsewhere (*91*).

Related to the issue of the food-derived bioactive peptides is the more general investigation about the 'metabolic fate' of the food proteins. Many static or dynamic model studies of the human digestion are trying to assess which protein domains survive the harsh hydrolytic events of the gastrointestinal passage. The majority of these studies suffer from the limits imposed by the *in vitro* conditions employed, which in any case differ from the *in vivo* physiology. Nevertheless, many models of the gastrointestinal digestion have tried to mimic as close as possible the physiological digestion allowing to identify some peptides that most likely survive digestion.

Some authors have described the identification of potentially bioactive peptides in extensively proteolyzed food products, such as the hypoallergenic infant formulas (*92*). In particular casein phosphopeptides, which are supposed to have mineral carrier properties, exhibit certain stability against processes of simulated digestion (*93–95*).

On the other hand, peptides more resistant to digestion are those potentially involved in eliciting the immune responses in IgE-mediated food allergies or in intolerances such as coeliac disease (*93,96*). These observations also confirmed that resistance to proteolysis is a valuable criterion to predict protein allergenicity and to select potential antigenic determinants (epitopes) of the immune responses (*97,98*). Peptides that preserve the allergenic potential of the parent proteins are expected to occur in fermented dairy products such as yogurt (*99*).

Prolamin-derived epitopes, potentially involved in triggering coeliac disease, have recently been identified in beer as the result of 'a proteolytic selection' that heavily degrades both the metabolic proteins and the storage prolamins from barley (*100,101*). The characteristics of allergenicity and intolerance of food proteins and derived epitopes have recently been reviewed (*97,98*).

Food Lipidomics

In general, lipidomics is more than just the comprehensive characterization of lipid molecules in a biological system, but it also includes the understanding of the functions of lipids within a biological system, in consideration of their primary role in cell signalling, membrane architecture, transcriptional and translational modulation, cell-cell and cell-protein interactions, and response to environmental changes over time. The complete definition of these aspects is obviously a very challenging task, but the basic knowledge of lipidomics entails the systematic characterization of the lipid components of a given system.

The concept underlying food lipidomics only slightly differs from the general aspect of lipidomics, taking into account that lipids, once regarded simply as energetic reservoirs, do have biological and nutritional roles, many of which are now well established. Furthermore, in the majority of foods the lipids and their interaction features drastically affect the overall physical characteristics, such as flavour, texture, taste and appearance.

If compared to proteomics and genomics, the lipidomic analytical methodologies are far from being standardized, primarily because of the extraordinary structural diversity of the molecules that are catalogued among lipids. The definition of lipids as biological compounds that have a hydrophobic nature and are in most cases soluble in organic solvents (*102*) fails to embrace the totality of molecules that are normally considered to belong to this class. The specific profiling of lipid subclasses characterized by specific structural features has made definitions such as 'phospholipidomics' (*103*), 'sphingolipi-

domics' (*104–106*), 'glycolipidomics' (*105*), 'steroidomics' (*107*) and even 'endocannabinoidomics' (*108*) appear in the literature.

The lipid profiling which is at the basis of lipidomics makes now extensive use of the 'omic' technologies and in particular MS. While the analysis of fatty acids remains strict domain of GC and GC-MS based techniques, although laborious and time consuming, atmospheric pressure chemical ionization (APCI) (*109*) and ESI (*110*) are currently considered to be the methods of choice in lipidomics.

The first analyses of triacylglycerols (TAGs) (as ammonium or sodium ion adducts) by direct infusion ESI-MS/MS date back to little more than 20 years ago (*111*). Since then, the impressive technical advances in MS have contributed inarguably to push forward the knowledge in lipidomics. Two basic strategies have been developed for the MS-based lipidomics, namely: (*i*) the focussed or targeted lipidomics that targets a single lipid or a lipid class, and (*ii*) the high-throughput global lipidomic approach that aims to a complete profiling of the components of a lipid mixture. Complex mixtures can be analyzed by HPLC coupled to ESI-MS and MS/MS or directly infused into the mass spectrometer. This latter approach has been referred to as 'shotgun' lipidomics (*112*). Shotgun lipidomics is particularly effective for polar lipids, such as phospholipids, due to the tendency of these compounds to readily undergo protonation or deprotonation, so permitting to carry out analyses in both positive and negative ion modes. The major concerns in shotgun lipidomics are the ion suppression and matrix effects that can be detrimental when non-polar or low-abundance lipids are to be detected. Lipids can undergo fragmentation even under mild conditions of ionization. Fragmentation can be structurally informative and can contribute to the ultimate structural assignment of lipids. The ionization aspects and the fragmentation routes for the various classes of lipids have been brilliantly reviewed (*113*). Several databases have been built for the purposes of shotgun lipidomics. However, because a universally accepted classification scheme of lipids is still missing, databases differ in their intent and organization, providing different or complementary informative levels. A recent list of on-line lipidomic resources is reported by Hou *et al.* (*114*).

Identification and quantitative evaluation of TAGs of several edible oil and fats, including palm oil, cocoa butter, tallow, lard and chicken fats, have been accomplished by HPLC coupled to APCI-MS (*115*). Positive ion mode high resolution ESI-FTICR-MS for the detection of DAGs (diacylglycerols) and TAGs provides fingerprints that allow a straightforward differentiation of vegetable oil (*116*). The same technique can be applied in the negative ion mode to assign oil by monitoring fatty acids and tocopherols. Both positive and negative ion mode profiling by direct infusion ESI-Q-TOF-MS allows to establish classification, quality, adulteration, and ageing of vegetable oil (*117*).

Also, HPLC-APCI-Ion Trap MS/MS was effective in disclosing adulterations of olive oil with foreign vegetable oil (*118*). The analytical platform was also validated in order to determine the relative ratio of positional regioisomers of mixed TAGs. However, a comparative study of APCI, ESI and negative ion chemical ionization (NICI) LC-MS methods for the analysis of TAGs from lard, rapeseed and sunflower oil questioned the reliability of LC/APCI-MS/MS for the quantitative determination of regioisomers (*119*). The same authors have later developed new UPLC/APCI-MS/MS strategies for the quantitation of regioisomeric TAGs in vegetable oil (*120*). Also for APCI, non-aqueous HPLC systems were tested in comparison with MALDI to characterize castor oil, which is particularly rich in hydroxylated TAGs (*121*).

The direct infusion multi-stage ESI-MS experiments and RP-HPLC-ESI-MS/MS approaches were compared, using linseed oil as a source of TAGs. The combination of both approaches permitted the identification of 26 TAGs and to discriminate isobaric regioisomers (*122*). Complex TAG mixtures from marine oil, such as cod liver oil, have been characterized and profiled by LC-ESI-IT-MS/MS (*123*). The technique also offered TAG fingerprints that clearly distinguished vegetable from marine oil, confirming itself a powerful technology that could be of great effectiveness for authorities and industries (*124*).

The glycerophosholipid and sphingolipid (sphingomyelin and ceramides) composition of food has attracted interest in consideration of their nutritional importance. ESI-MS/MS methods are for instance ideal to face the structural diversity of sphingomyelin-related compounds. Thus, sphingomyelin compounds have recently been profiled and quantified in potatoes (*125*), meat (*126*) and human milk using hydrophilic interaction liquid chromatography (HILIC) coupled with ESI-MS/MS (*127*). These data can provide information to understand the nutritional aspects of sphingomyelin and, in the case of breast milk, to design humanized and functional substitutive infant formula.

MS methods, also exploiting multiple ion reaction monitoring (MRM) and neutral loss strategies for structural assignment, for the analysis of sphingoids have been well established (*128*).

Growing evidence demonstrates that MALDI-TOF-MS provides experimental handiness and analytical reliability, thereby representing itself as a valuable alternative or complementary tool (*129*). Soft ionization techniques do not require derivatization prior to analysis and allow to maintain the integrity of the molecules, preserving relevant structural information. Because of several advantages that include minimal sample handling and short analysis time, MALDI-TOF-MS is increasingly used to profile food phospholipids and neutral lipids. For instance, phospholipids and TAGs from hen egg yolk have recently been profiled by MALDI-TOF-MS, utilizing several innovative matrices (*130*). Bovine milk fat and deposit fat were also profiled using 2,5-dihydroxybenzoic acid (DHB) as a matrix and several signature molecular species were identified to easily discriminate these animal-derived fats (*131*). With a simple expedient, which requires a few minutes at the stage of sample preparation consisting in the addition of bromine to double bonds, saturated TAGs were differentiated and analyzed without the interference of unsaturated species that shifted at higher molecular mass by 160 Da per double bond (*131*). The full hydrogenation of TAGs followed by MALDI-TOF-MS profiling has also enabled to discover less abundant TAG families containing short- or very

long-chain fatty acids in both milk fat (*131*) and fish oil (Picariello *et al.*, unpublished data). Vegetable oil can be in general discriminated by MALDI-TOF-MS on the basis of the TAG profiling (*132*). Information about the geographical origin of olive oil has also been obtained (*133*). The gross silica chromatography fractionation in polar and apolar lipids also allowed to characterize a high number of compounds in thermo-oxidized vegetable oil (*134*) formed because of hydrolytic oxidation (DAGs) and of radical decomposition of unstable hydroperoxides (β-scission products, core aldehydes, TAG oligomers). Of particular interest is the possibility to elucidate the fatty acid regioisomerism by high energy collision-induced dissociation (CID) fragmentation analysis using MALDI-TOF/TOF-MS/MS instruments, as made on synthetic TAGs and from plant oil (*135*).

This latter is an example of the monitoring of the evolution of lipid fraction in foods, in this case due to a thermal treatment. Lipids also change significantly during storage and ripening, affecting sensorial properties of food. The study of lipolytic phenomena has remained, up to now, practically confined to the application of GC and GC-MS methods. The informative level obtained by MALDI-TOF-MS was in this case comparable to that of APCI- and ESI-MS-based techniques (*136,137*). Under strictly controlled experimental conditions, MALDI-TOF-MS provides quantitative responses that are consistent with GC-based determinations, as demonstrated by a focussed comparison of the two methods applied to the evaluation of TAGs in cocoa butter (*138*). However, minimizing in source TAG fragmentation and rendering homogeneous the matrix/analyte crystallization, for instance through a pre-coated film substrate of nitrocellulose, and in the presence of adequate internal standards, MALDI-TOF-MS can be reliably utilized for quantitation purposes (*139*).

In spite of their low molecular mass, saponified fatty acids have been detected by MALDI-TOF-MS as doubly alkaline metal cationized species, using as matrix meso-tetrakis(pentafluorophenyl) porphyrin, which ionizes in a spectral region outside of the range of fatty acid molecular masses (*140*). The advantages and the drawbacks of the use of MALDI-TOF-MS for profiling polar and apolar lipids, with the emphasis on the oil and fat of nutritional interest, are excellently summarized in several reviews (*129,141,142*).

Two additional analytical parameters, high sensitivity and specificity (mass resolution), account for the advantages of using mass spectrometry in lipid analysis. High-resolution mass spectrometry allows the identification of previously uncharacterized lipids and discrimination between lipids with similar mass and chemical structures. However, some specific analytical targets cannot be addressed satisfactorily by MS alone. These include unequivocal assignment of structures, including regioisomerism and position and configuration(s) of double bond(s), which remain difficult to be readily assigned by MS/MS (*143*). However, double bond can be assigned by high energy CID.

Positional regioisomers can be discriminated by MS/MS, as demonstrated by the APCI or ESI-MS/MS applications to TAGs of milk fat from different species (*144*) and to vegetable oil (*120,145*). Nevertheless, ^{13}C

NMR (nuclear magnetic resonance) has been shown to be more reliable in assigning the preferential *sn*-position of specific fatty acids on the glycerol backbone (*146,147*).

Carotenoids and xanthophylls have also been detected by LC-ESI-MS as radical cations [M$^+$] using trifluoroacetic acid as post-column oxidant (*148*) and recently in a more specific way by LC-ESI-MS/MS (*149*). Hyphenated techniques such as HPLC coupled to DAD and ESI-MS detection have been exploited to determine geometric isomers of carotenoids in mature grapes (*150*).

MS-based screening can be utilized for the detection of steroid species, as demonstrated by the relevant high-throughput determination of a panel of 34 residual steroids in bovine muscle (*151*). Related to this last issue is the determination by MS of vitamin D (*152*), and specifically vitamin D3 (cholecalciferol) in foods such as skimmed milk, orange juice, multigrain cereals, and salmon (*153*). Phytosterols, which are believed to bring nutritional benefits and are therefore used as dietary supplements, can also be detected by MALDI-TOF-MS, as demonstrated by the determination of β-sitosterol, campesterol, and stigmasterol in pomegranate oil (*154*). The technique of MS-imaging of tissue sections based on the profiling of lipids has found scarce application in food-omics so far.

Food Glycomics

The glycans of glycoproteins play multiple roles in biological systems and are dynamically remodelled in order to modulate the (glyco)protein functions. Biosynthesis of glycans is a non-template driven process that is not ruled by the gene control. As a consequence, glycans are characterized by a higher degree of inherent structural diversity than proteins or nucleic acids. The determination of both structures and structure/function relationships is made difficult by the heterogeneity of the monosaccharide units in glycans and by complex branching patterns. Multistage MS combined with up-to-date separation methodologies has become one of the most powerful and versatile techniques for the structural characterization of glycoconjugates. In foods, both N- and O-linked glycoproteins can affect not only the technological properties of proteins, but also the digestibility and the potential allergenicity (*155*). For instance, several plant N-glycans containing α-1,3-fucose and β-1,2-xylose have been described as IgE-binding epitopes (*156–158*). Carbohydrate moieties of glycoproteins can also indirectly increase the allergenic potential by increasing protein resistance to proteolysis (*159,160*). In consideration of the large range of biological actions exerted by the glycan moieties of glycoproteins, glycoproteomics is emerging as a branch of proteomics promoting the development of dedicated applications that are by now well established while several advances are still progressively appearing. Protocols, methodologies and technologies for facing the challenges of glycoproteomics have already been reviewed (*161*).

Further to the characterization of food glycoproteins, a recent emerging field in nutritional research is the study of food glycans, in particular oligosaccharides. To the best of our knowledge the topic of the food glycomics

has not been specifically focussed on by any review so far. Food-derived oligosaccharides are generally non-digestible ingredients, therefore they are not absorbed in the upper part of the gastrointestinal tract (*162*). However, they are utilizable by gut microflora, which in turn cleaves oligosaccharides to smaller molecules available to the host. The oligosaccharide digestion in the colon produces acetic and lactic acids that, by lowering the pH, inhibit the growth of pathogenic microflora. Due to the stimulating activity for the growth/activity of the beneficial microflora in the colon, oligosaccharides and glyconjugates are considered as prebiotics (*163*). Dietary glycoconjugates and oligosaccharides are supposed to be involved in dental caries prevention, enhancement of passive immunity, mineral transport, and regulation of blood glucose in diabetics. Structural properties of functional oligosaccharides have been summarized recently (*164*).

Under the perspective of food glycomics, intended as the profiling of glycoconjugates in food products, several MS-based applications can be quoted. For instance, a very large number of plant glycomic characterizations have been reported (*165*).

Fructans from onion bulbs were characterized by MALDI-TOF-MS after fractionation by high-performance anion-exchange chromatography (HPAEC). To this purpose, MALDI-TOF-MS was exploited for first time to analyze entire plant tissues or parenchyma cell layers (*166*). A similar MALDI-based characterization was carried out for garlic (*167*). Negative-ion mode MALDI-TOF-MS, used for the characterization of storage, neutral oligosaccharides extracted from Jerusalem artichoke (*Helianthus tuberosus*), red onion, and wheat, was demonstrated to be effective also in discriminating neutral carbohydrates containing reducing ends from non-reducing ones (*168*). MALDI-TOF-MS analysis of these same vegetable oligosaccharides and, additionally, of glycose syrups from potatoes was demonstrated to be more powerful than the classical chromatographic approaches (*169*).

In addition to the identification, MALDI-TOF-MS was also utilized for the quantification of fructooligosaccharides (FOS) of red onions, shallots, and elephant garlic. For quantitative purposes THAP (2',4',6'-trihydroxyacetophenone) appeared to provide more reliable data than the most commonly utilized matrix DHB. FOS and inulin from plants have also been analyzed and quantified with high resolution FTICR mass spectrometry (*170*). In most cases, pre-fractionation of the water or low ethanol plant extracts with hydrophilic interaction liquid chromatography (HILIC) or porous graphitized carbon solid phases is crucial for carrying out accurate MS analysis of purified glycans (*171*).

Oligosaccharides from legumes, with special regard to the nondigestible α-galactosides, have been profiled by amino-bonded silica columns used for HPLC coupled to refractive index detector (*172*). Alternatively, glycans can be derivatized with phenylhydrazine to be detected by UV. The derivatization also enhances the detection by ESI-MS and MALDI-TOF-MS (*173*). However, ESI-MS and MS/MS can be used as a more specific, informative as well as sensitive detector for a global analysis of underivatized oligosaccharides. For instance, beer maltooligosaccharides have been analyzed by LC-ESI-MS/MS (*174*).

Arabinoxylans from wheat have been characterized by both MALDI- and ESI-MS/MS, also obtaining information about monosaccharide linkages after permethylation (*175*). On the other hand, the complex structures of arabinogalactans of glycoproteins in wheat flour have not been elucidated yet (*176*). In any case, for a full elucidation of glycan linkages and branching, MALDI and ESI have to be complemented by GC-MS analysis of derivatized sugars and/or by NMR advanced techniques. Advances in resolving glycan structural isomers have been obtained by gas phase ion mobility separations (*177*) or by energy-resolved MS (*178*). MS is amenable to the profiling of complex glycoconjugates. For instance, galactolipids, along with lipids and oxylipins in potato tubers (*179*), and in *Ipomoea batatas* leaves (*180*) were characterized by ESI-MS/MS. Glycolipids and glucocerebrosides from red bell pepper (*Capsicum annuum*) have been profiled by HPLC-APCI-MS/MS (*181*). Tetragalactosyl DAGs have been characterized by multistage MS in oat kernels (*182*). It is to be underlined that several galactolipids are believed to possess *in vitro* and/or *in vivo* anti-tumour promoting as well as anti-inflammatory activities. A huge number of food-derived glycoside metabolites, not overviewed here, have been profiled by MS techniques.

MS techniques, including high resolution FTICR-MS, have been applied for the analysis and structural characterization of neutral oligosaccharides of human milk (*183*). Already more than ten years ago the resistance of human milk oligosaccharides to the gastrointestinal digestion was assessed by MALDI-based techniques (*162*). Throughout the decade, several MS-based strategies to comprehensively characterize native glycans in milk (*184*), also including non-neutral species, have been developed (*185*). Glycans have been identified, profiled and monitored during lactation not only in human milk but also in milk from other species including bovine (*186*), swine (*187*) and primates (*188*). These studies have allowed to enrich the list of milk oligosaccharides and also to determine several of their functional properties. In particular, the bacterial consumption of oligosaccharides proved to be strain specific (*189,190*), thereby suggesting a means for modulating the prevalence of specific probiotics to some extent. Cheese whey was expectedly demonstrated to be a source of nutritionally relevant oligosaccharides that have been profiled by MS (*191*).

Numerous bioinformatic tools support the research in glycomics and the storage of the achieved results. A collection of web resources for glycomics is reported by Raman *et al.* (*192*).

Food Metabolomics and Metabonomics

Food is the result of the interaction between ingredients and technological processes. It can be regarded as the product of the modifications induced on the raw materials by the concurring action of physical, chemical and biochemical agents during the production process. As a consequence, an enormous variety of compounds is generated or modified, which are responsible for the quality (texture, flavour, odour, colour and shelf life) and nutritional value (vitamins, antioxidants, and nutrients) of the final product. Therefore, food can be fully defined

in terms of the metabolic profile of the material concerned at a particular time (metabolome).

Metabolomics is defined as the comprehensive analysis of the metabolome without particular bias to specific groups of metabolites. On the other side, metabonomics is a term mostly used in the biomedical field to describe the fingerprinting of dynamic biochemical changes induced by external agents on biological systems. In contrast to transcriptomics and proteomics, which rely to a great extent on genome information, metabolomics is mainly matrix independent, which means that it can be applied to diverse samples, foods included, without a laborious re-optimization of protocols. Most metabolites fall in the low molecular mass range. The various technologies used for metabolomic/metabonomic analysis have been excellently reviewed (193,194). A wide range of technologies are based on spectroscopy and high-resolution chromatography linked to a range of detection methods (mainly MS, but also, more recently, NMR spectroscopy, this last especially in metabonomic applications). Well-established MS ionization techniques such as atmospheric pressure chemical ionization MS (APCI-MS), as well as the newest atmospheric pressure photoionization MS (APPI-MS), are finding large application for their ability to analyze low molecular mass molecules, which include the major range of metabolites of interest (195). A complex range of other instrumentation and their combinations, *e.g.* LC-NMR, is going to be deployed in the near future (196).

From the metabolomic perspective, most foods can be considered as complex chemical mixtures consisting of various metabolites and chemical additives in a solid, semi-solid or liquid matrix. Some foods consist of just a few different compounds (artificial energy drinks, soft drinks, purified vegetable oil), while other foods consist of hundreds of compounds (milk, cheese) and still others may have thousands of compounds (fruits, meats and most prepared foods). In food technology, metabolome profiling is gaining great importance in many directions. These include: tracing the evolution of a product from the starting materials to the finished foods; evaluating the effects of either desired or undesired microbial action on food quality; monitoring the production or persistence of functional and bioactive compounds; and, in general, measuring the action of technological treatments (physical, chemical, enzymatic) on the development of rheological and sensory characteristics (197). Examples in dairy, cereal, wine or meat products are depicting the metabolomic fingerprint of these products.

Because of the increasingly globalized nature of food production and processing, along with the growing concerns over food safety and food quality, more stringent and precise food monitoring systems are being developed in these years. These approaches are being applied to the control and detection of foodborne microorganisms. Traditional means of controlling microbial contamination and safety hazards in foods include freezing, blanching, sterilization, curing, and the use of preservatives. However, the developing consumers' request for 'natural foods', as indicated by the strong growth in sales of organic and chilled food products, has resulted in a move towards milder food preservation techniques. This raises new challenges for the food industry. Metabolome profiling has

recently led to fast and sensitive detection of microorganisms affecting meat quality and safety during processing and storage (198,199). More accurate description of the contaminating microorganisms has been achieved by integration of proteomics with peptidomic and metabolomic methodologies able to provide either structural or quantitative identification of specific metabolites produced by the various spoilage microorganisms (198,200, 201).

Biogenic amines, which derive from bacterial decarboxylation of amino acids during decomposition, are well known molecular indicators of spoilage. In a very recent investigation an UPLC-Orbitrap-MS method has been demonstrated as robust and effective for the one-step determination of a panel of eight biogenic amines in tuna (202). A similar HPLC-ESI-MS approach has already been developed for the quantification of biogenic amines in cheese (203). It can be seen that these methods are being integrated to design sensitive sensors on a microchip surface for automated detection.

Another crucial aspect of metabolomics in food technology is the characterization of compounds which give food its peculiar taste, odour, flavour, colour and in general the sensory characteristics highly appreciated by consumers. Data available on the key food taste compounds are abundant but contradictory, particularly in the case of fermented products (meat and dairy products, wine and beer) where the flavour compounds are generated by a complex series of metabolic and technological processes. The taste-active molecules which differentiate the many different types of cheese are, for instance, the products of a cascade of lipolytic and proteolytic pathways that produce a large variety of compounds imparting typical sensory characteristics to the final product. The comprehensive spectrum of sensory-active, low molecular mass compounds, the 'sensometabolome' (204), reflects the sensory appearance and triggers the typical smell and taste of food products. The goal of sensometabolomics is to catalogue, quantify, and evaluate the sensory activity of metabolites that are present in raw materials and/or are produced upon food processing such as thermal or high-hydrostatic pressure treatment and fermentation. The sensometabolomic approach is based on MS analysis combined with analytical sensory tools, and has been applied recently for identification of the aroma compounds in wine (205). A class of olfactory-active metabolites recently emerged in wine are thiol compounds, which may play an important role in the flavour development of a wine, although they are normally present only at trace levels. Volatile thiols are of particular importance for the varietal character of wine made from Sauvignon blanc cultivar, imparting passionfruit, grapefruit, gooseberry, blackcurrant, lychee, guava and box tree typical fragrances. These compounds have also been identified in other wines made from Riesling, Colombard, Semillon, Cabernet Sauvignon and Merlot grapes in various concentrations. It is believed that the precursors of sulphur flavours are produced in grape as odourless glutathionyl-conjugates (see scheme in Fig. 3). Aromas are then released from precursors during fermentation, probably by action of a specific β-lyase. Recently it has been reported that several compounds (including 4-methyl-4-mercaptopentan-2-one, a molecule which plays a
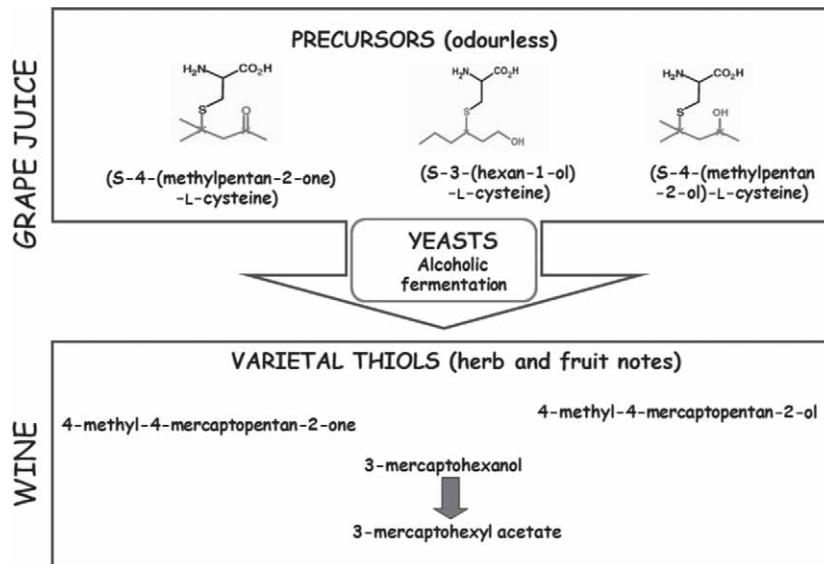
Fig. 3. Volatile thiols such as 4-methyl-4-mercaptopentan-2-one, 4-methyl-4-mercaptopentan-2-ol and 3-mercaptohexanol are of particular importance to the varietal character of wine made from Sauvignon blanc, conferring the passionfruit, grapefruit, gooseberry, blackcurrant, lychee, guava and box tree typical fragrances. The precursors of sulphur flavours are produced in grape as odourless glutathionyl-conjugates. Aromas are then released from precursors during fermentation, probably by the action of a specific β-lyase

key role in the aroma of Sauvignon) derive from S-conjugated compounds of cysteine or glutathione. By metabolomic analysis (Fig. 4), correlations were established among the possible changes in the composition of the analytes of interest and the technological choices made in the experimental wines (different strains of yeast, different adjuvants and additives, *etc.*) for each specific case (*206,207*). In this way, a class of molecular markers of quality have been identified, closely related to the varietal characteristics, geographical origin and specific choices of the technological process, and sensory aspects.

Food Interactomics

Interactomics is a discipline at the intersection of bioinformatics and biology studying both the interactions

and the consequences of those interactions between and among proteins, and other molecules within a biological system (*208*). The network of all these interactions is called the interactome. The field of interactomics is now rapidly expanding and developing: over 90 % of proteins in *Saccharomyces cerevisiae* have been screened and their interactions characterized, making it the first interactome to be nearly fully specified (*209*). With respect to the field of nutrition science, the recent 'omic'-based developments also supported the hypothesis of a correlation between human intestinal microbiota and health status, causing significant interest in microbe-host interaction studies (*210*). A further study proved the modulating effects on the immune system of *L. plantarum* strains and identified genes supposedly responsible for the stimulation of pro- or anti-inflammatory immune responses in the gut
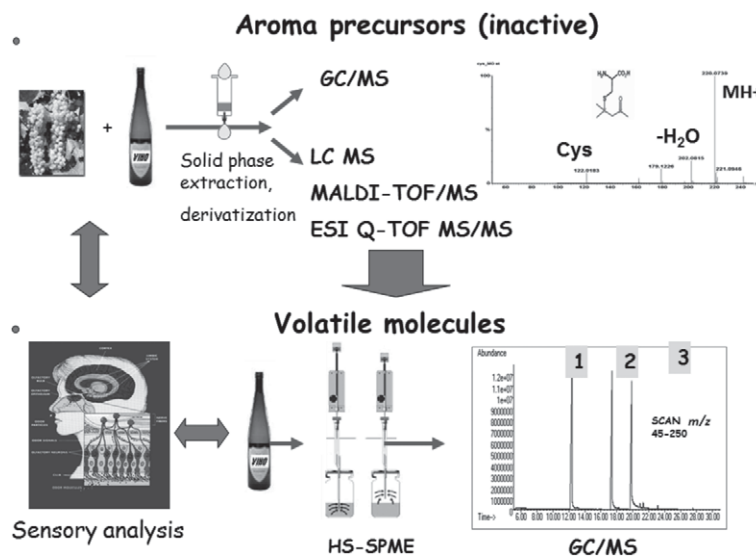
Fig. 4. Sensometabolomic scheme of analysis of volatile thiols and their odourless precursors in Sauvignon blanc grape and wine

(*211*). The characterization of the interaction mechanisms described for *Lactobacilli* and *Bifidobacteria* (*212*) shifted the traditional paradigm from analyzing pathogens to that involving commensal and probiotic bacteria.

Preliminary maps of human and bovine milk protein interactomes have been built up by using integrated *in silico* studies, multiple pathway and network analyses (*213*). As expected, most of the milk proteins were grouped under pathways/networks/ontologies referring to nutrient transport, lipid metabolism and immune system response, as well as novel functional families which included proteins involved in the induction of cellular proliferation processes.

With regard to the technological aspects, it is known that, because of their structural properties, proteins may establish either covalent or non-covalent interactions with other proteins or with different food components, including water, which results in changes in protein hydration, solubility, viscosity of solutions, film formation, gelling, and adsorption at the interface between aqueous and lipid phases. Over time, the integrated approach based on various 'omics' for food peptide and protein characterization, all of which rely on MS analysis, are accelerating the development of functional food products not limited to food industry. For example, the capacity of MS techniques to analyze supramolecular complexes is opening new scenarios in the understanding of the protein--protein, protein-carbohydrate, and protein-lipid interactions, which in turn determine the rheological properties of complex matrices; this is the case, for example, of wheat dough and of its industrially designed non-conventional substitutes directed to intolerant patients, where transglutaminase-modified non-gluten proteins are being tested (*214*).

Many plant metabolites bind dietary proteins and gut enzymes in either reversible or non-reversible way. In food technology, polyphenols are involved in the formation of precipitates in beverages and in modifying rheo-

logical and organoleptic properties (*215*). These properties are largely due to the complex, noncovalent interactions established with food matrices, which therefore assume a great significance from the technological point of view. For instance, polyphenols are responsible for the astringency feel of many beverages and foods. This is thought to be caused by the interaction of condensed polyphenols, tannins, with basic salivary proline-rich proteins (PRPs). It is widely assumed that the molecular origin of astringency is the precipitation of PRPs following polyphenol binding and the consequent changes to the mucous layer in the mouth. The analysis of these interaction complexes has been made possible only very recently by the introduction of soft mass spectrometry ionization techniques such as ESI-MS and ion mobility MS (IMS) (*216*). The measurements following ESI in 'native' conditions have supported the use of IMS to explore conformational adaptability of intrinsically disordered proteins bound to their targets in complex mixtures. By these approaches, the interactions between a human PRP and a model of wine and tannin were characterized (*217*).

Protein interaction with phenols also determines the quality of barley in malting and brewing end-uses. In this regard, water-soluble barley proteins play a major role in the formation, stability, and texture of head foams. A cohort of 'omic'-based studies has recently been dedicated to the identification of haze- and foam-forming proteins and of their mechanism of action, with the aim of elucidating and controlling their occurrence during beer production and storage (*218,219*).

In these studies, the influence of barley malt protein modification on beer foam stability and their relationship with the barley dimeric α-amylase inhibitor-I (BDAI--I) as a possible foam-promoting protein was investigated (*220*). 2-DE and MS were combined to highlight the barley proteins that resist the heating treatments during malting and brewing processes. The involvement of these proteins in the quality of beer foam can now be evaluated and technological improvements be adopted (*221*).
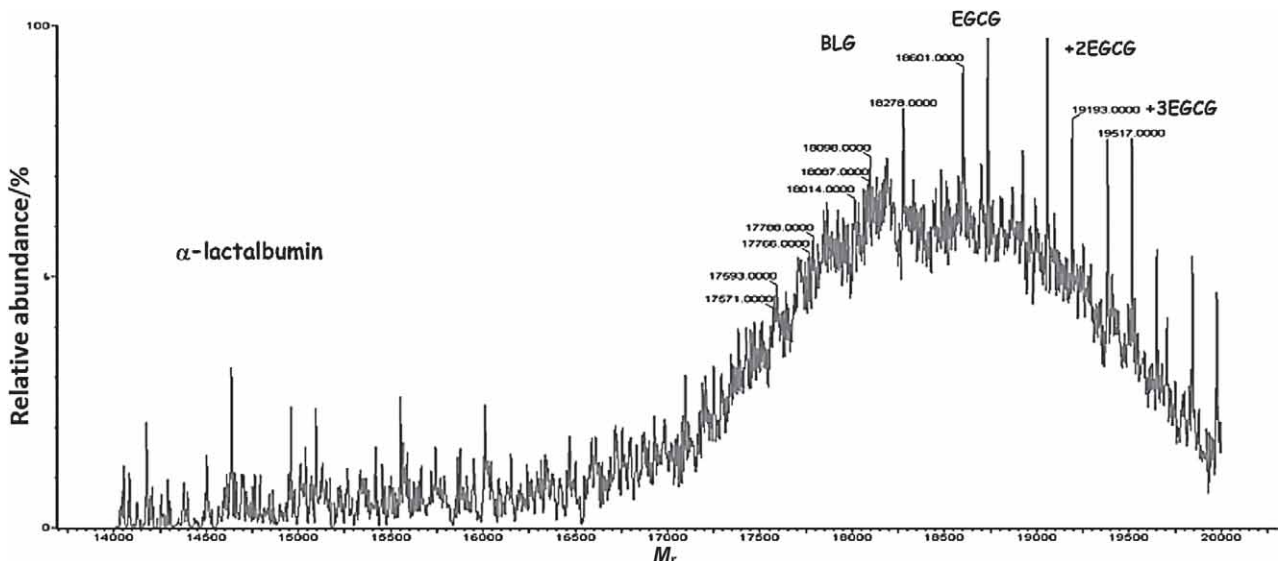

Fig. 5. Epigallocatechin/β-lactoglobulin (EGCG/BLG) non-covalent complexes in a mixture of bovine whey proteins and black tea analyzed by ESI-MS analysis under non-denaturing conditions. The two genetic variants of bovine milk BLG (A and B) originate from two series of molecular complexes

From the nutritional point of view, the polyphenols in tea, coffee and cocoa are considered a source of bioactive components with important antioxidant and health-promoting effects for consumers. In combination with milk or milk powders, these ingredients are used for preparation of breakfast food beverages, cocoa drinks, milk and chocolate bars. It is believed that in these products the nutritional value and bioavailability of polyphenols are lowered because they are sequestered by interaction with milk proteins. These statements may find a structural confirmation by using ESI-MS and IMS to detect weak protein complexes (*222*). In Fig. 5 the molecular aggregates formed by interaction between β-lactoglobulin (BLG) and tea polyphenols are analyzed by ESI-MS in native conditions. The ability of BLG to bind organic molecules noncovalently has recently been proved by applying the same approach to the identification of the endogenous ligands of this still enigmatic milk whey protein (*223*).

In a recent study the interactions between plant polyphenols with milk β-casein and their effects on the surface properties at the air/liquid interface have been monitored by combining rheological data with the information provided by ESI-MS and light scattering on the formation of noncovalent polyphenol-protein complexes (*224*). Covalent structural modifications in milk whey proteins induced by phenols have been characterized by MALDI-TOF-MS (*225*). This integrated approach could probably be used as a basic methodology in the future, also applying the novel IMS for the studies of supramolecular complexes (*216*) in order to evaluate the changes in food functional properties (odour binding, nutrient transport) induced by the formation of protein complexes, but also to use the binding protein of milk proteins such as β-casein or BLG to design new drug carriers for the pharmaceutical industry.

Perspectives

The 'omic' sciences are ideally positioned to be used in most areas of food science and technology and in nutrition research including food component analysis, food processing, food quality/authenticity assessment, food additives and contaminant analysis, or food consumption monitoring for intervention studies. The actual and potential contributions of the various 'omics' to food science are so extended that they are hard to be adequately highlighted. However, this impact is still limited by several factors: instrumental cost, technology, and software/ database availability. Costs either in terms of initial investments or managements still limit a wider diffusion of the newest generation MS and NMR instruments to research as well as industrial laboratories. In terms of technology, it appears that significant improvements need to be made to make target compound detection and quantification technology more robust, automated and comprehensive. In the case of food metabolomes and interactomes, while promising advances have already been made recently, current techniques are only capable of detecting perhaps a fraction of the relevant compounds or functional links. As a result, no food metabolome or interactome at present has been completely characterized yet. It is expected that the advances and future developments of protein array technology, which will be largely driven by MS-derived structural information, will ensure more rapid and accurate assessment of food safety for the consumer. For all these reasons, employment of the novel MS techniques in food proteomics is expected to increase during the next years.

Updated and dedicated software and databases, similar to those already available for proteomes and peptidomes, with more reference spectral and chromatographic collections of metabolites, food components and phytochemicals need to be developed for food lipidome, glycome, and (senso)metabolome. While these accomplishments may take years to be achieved, hopefully the food science community will find a way to coordinate its activities to complete these efforts. Over time, the integrated approach based on various 'omics' for food characterization should accelerate the development of functional food products as well as increase the knowledge to develop novel foods and ingredients for various applications, not necessarily limited to food industry.

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