Integration of multi-omics data for biomarker identification of food safety and quality

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In the last decades, consumers' interest in healthy, fresh and convenience foods has greatly increased. Due to globalization, food scenario is rapidly changing and moving from the consideration of food as a mere source of energy to a growing awareness on its importance for health and particularly in reducing the risk of diseases. The continuous advance in the field of molecular biology allowed setting up efficient and universal omics tools to address food safety and quality. In this review, we look at the current progress of applying omics technologies to identify biomarkers of food safety and quality. We consider the application of a multi-omics approach integrating proteomics, metabolomics, metagenomics, transcriptomics (via systems biology), in characterizing the composition of food products along the food supply chain. The combination of the above omics approaches allows us to define a sort of molecular labeling of food or biomarkers that are easily understandable by the operators involved in the food sector.

1. Introduction: the challenge of omics in the field of food safety and quality

The globalization of the food market has raised major concerns in terms of safety and quality of foods due to the worldwide increase in the movement of foodstuff and related raw materials worldwide as well as the shipments of multiple and processed ingredients from different parts of the globe [1]. Food chain, food safety, and food-processing sectors face new challenges due to the globalization and the continuous changes in the modern consumer preferences. In addition, the gradual increase in microbial resistance, changes in climate, and incorrect food handling remain a pending barrier for the efficient global food safety management [2]. An important aspect of food science is the need to trace food products along the entire food supply chain and to ensure all the safety, nutritional quality and acceptability issues related to of the delivered products. Although at present half of planet's population doesn't have access to a sufficiently nutritious diet [3], in the countries where economic growth is present there is a demand and an emerging trend for nutritiously and healthy foods, as well as a rising consumer concern for food safety and quality. In fact, food safety refers to all those hazards, whether chronic or acute, that may have a negative impact on the health of the consumer, while food quality mainly refers to all other attributes that influence a product's value to the consumer, including contamination, discoloration, off-odours, origin, colour, flavour, texture, and processing method of the food. In the past two decades, our ability to evaluate the food safety and quality has radically changed through the development of high-throughput, omics technologies [4] (Figure 1 and Table 1).

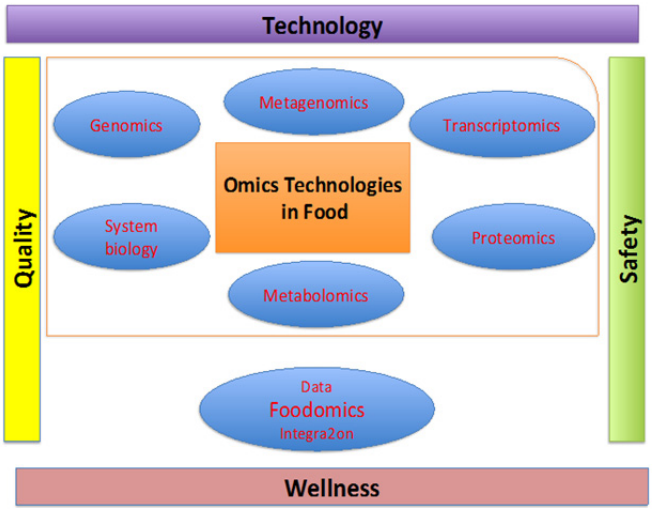

Fig. 1 A new comprehensive approach to food quality and safety assessment.

Table 1 The role of omics technologies for the detection of food quality and safety.

Omics approaches	Molecules of interest	Description	Approaches and technologies	References
Genomics	DNA	Genomics is the systematic study of the structure, function, and expression of all the genes in a cell/ organism/sample	16S rRNA gene clone library, Quantitative PCR, Whole genome sequencing	[5], [6], [7]
Metagenomics	DNA	Metagenomics is defined as the direct genetic analysis of genomes contained with a cell/organism/sample	16S rRNA gene sequencing, Shotgun sequencing	[8], [9], [10]
Transcriptomics	RNA	Transcriptomics is the study of the total mRNA in a cell organism/sample	DNA microarray, RNA sequencing	[11], [12]
Proteomics	Proteins	Proteomics is the large-scale study of proteins, including their structure and function, within a cell/ /organism/sample	Peptide mass fingerprinting (PMF), MS/MS, LC-MS, MS, DIGE technology	[13], [14]
Metabolomics	Metabolites	Metabolomics is the study of global metabolite profiles in a system (cell, tissue or organism) under a given set of conditions	Fourier Transform Infra-Red Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), Mass Spectroscopy (MS) and High Resolution Mass Spectrometry (HRMS)	[15], [16], [17]
System biology	All	System biology is the systematic study of complex interactions in biological systems	Integration models	[18], [19]
Foodomics	All	Foodomics is the comprehensive, high-throughput approach for the exploitation of food science in the light of an improvement of human nutrition	High-throughput approach in the field of food chemistry, analytical chemistry, biochemistry, microbiology, molecular biology, food technology, clinical sciences, and human	[2], [20]

The advancement in sequencing technologies and various 'omics' tools has impressively accelerated the research in this area, presenting several advantages over traditional approaches [21]. In fact, compared to traditional methods, omics technologies appear to combine the benefits of relative simplicity, sensitivity, the speed of generating information and analysis of foods at various levels. By utilizing omics technologies, researchers can comprehensively compare food products at a molecular level, by analyzing the protein/gene expression/microbiome/metabolite components and composition changes during processing, storage, and transport. The *omics* approach has revolutionized the study of food allowing to develop suitable biomarkers for addressing food quality, authenticity, and safety issues, as well as to correlate all food components to the individual diet and the health. To achieve this goal, researchers involved in modern food science need to work within multidisciplinary teams in order to be able to face the huge complexity of this task and to rationally handle the huge amount of data generated by omics technologies (Figure 1). The integration of different omics technologies and the use of bioinformatic and advanced computational tools are key factors in the system-level understanding of relevant genes, variants, pathways or metabolic functions characterizing the food products and to monitor critical points in the food production/manipulation chain and the processes in the food industry.

2. Genomics and Metagenomics

The most common way of determining the composition of food-associated microorganisms has been through culturing methods that are based on the isolation and cultivation of microorganisms before their identification and typing. Genomic analysis to identify isolates to the genus or species level is more reliable and has greater discrimination than phenotypic methods. Among the plethora of molecular identification and characterization technologies available to date, the Whole Genome Sequencing (WGS) represents a significant tool in the area of food safety and food technology developments. This technique is being adopted by regulatory agencies around the world to identify bacterial isolates from foods, due to advances in sequencing technology (Figure 2). The entire genome sequences of numerous foodborne pathogens have been determined and the rapid and accurate detection and identification of foodborne pathogens are possible due to the many useful sequence analysis tools and software programs available in public WGS databases [22]. There are several examples of using sequencing for solving the epidemiological source of

foodborne microbial outbreaks [23]. In addition, the identification of bacteria at strain level by means of molecular typing tools, such as pulse-field gel electrophoresis (PFGE), ribotyping, and PCR-based techniques, is important for the purposes of surveillance and outbreak investigation. Anyway, all the above molecular methods that permit to identify and type food microorganisms require the isolation and culturing of microorganisms. Cultivation of microorganisms poses several limits and potential problems, which can be overcome by nucleic acid-based detection methods [9] [24]. Those limits include: i) the impossibility to detect most of the foodborne viral pathogens, ii) the presence of pathogenic bacteria in the viable but not cultivable state (VBNC), the ambiguous identification of pathogens due to the lack of selective media, iv) the possible long times necessary for the growth of the microorganism.

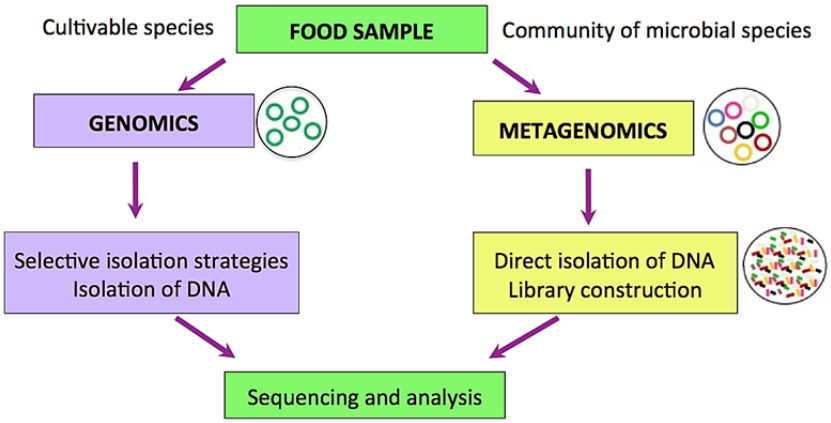

Fig. 2 Schematic representation of the differences between genomics and metagenomics.

Over recent decades, a great number of culture-independent methods have been developed that help overcome these problems; most of which have been used extensively in food systems [10]. Since the introduction of the DNA-based methods in microbial ecology, it became clear their potentiality in food microbiology, as a set of tools for fast and precise identification of target pathogenic organisms as well as for the detection of food quality [25]. Such DNA-based methodologies generally rely on specific DNA sequences (markers) that can be used for approving the quality and origin of raw ingredients. 16S ribosomal RNA (rRNA) gene sequences are commonly used to identify, quantify, and visualize microorganism populations in foods [26] because their genes consist of highly conserved domains interspersed with variable regions. Barcode sequencing of 16S rDNA performed on total microbial DNA from food samples enabled to evaluate the microbial diversity based on Operation Taxonomic Units (OTUs) composition as well as to assess their taxonomic status, and to further individuate biomarkers for their application in addressing quality, technology, authenticity, and safety issues. The application of specific DNA detection methods, as Real-Time PCR protocols, has been promoted and specific international agreements and standards have been released [27] [28]. In the last ten years, the advances in massive parallel sequencing or high throughput sequencing (HTS) technologies have opened a new perspective in food microbiology [29]. The development in metagenomic approach opens the way to previously unknown scenarios to detect microbial activities in microbes without requiring their cultivation since it gives the possibility to analyze the meta-community dynamics and to identify markers of food microbiota changes following the processing, treatments, transport and storage process. Among the most commonly reported HTS technologies are Illumina, where an efficient protocol for 16S rRNA gene-based analysis is the most widely used [30] but other technologies are on the market and used in microbial pathogen detection, as the 454 Roche pyrosequencing, the non-optical Ion Torrent device, and the single-molecule systems Nanopore-MinION and PacBio-SMRT (see for examples [31] [32] [33]). Those technologies may enable both amplicon-based sequencing (using 16S rRNA amplification primers targeting hypervariable regions) and untargeted metagenomic analyses (where sequence fragments from the virtually whole environmental DNA, including all the microbiome present in the sample, is reported). In this way, it is possible to detect the presence of specific microbial taxa and strains and the presence of functional genes in their genomes (e.g. toxin-encoding genes).

Metagenomics (i.e., the study of microbial communities sampled directly from their natural environment, without prior culturing) is currently applied primarily for the study of the microbiota composition from an ecology perspective (Figure 2). This technology offers a path to the study of their community structures, phylogenetic composition, species diversity, metabolic capacity, and functional diversity. In food science, it is essential to evaluate not only the species diversity of microbial communities but also to analyze how the species structures of those communities change over time and space.

Metagenomic sequencing can widen our perception of food microbiology, from a focus on a (small) panel of microbial taxa to an ecological interpretation of the food microbiome, as a “living” matter, where microbes interact each other and may possibly establish antagonistic interactions, which in turn, can limit the growth of undesirable strains [9]. Both the targeted one (“16S metagenomics”) and the untargeted one (“metagenomics *sensu stricto*”)

require several steps for data generation and processing. In particular, DNA must be extracted from the matrix under study and should be of enough quantity and quality for its successive PCR amplification (for targeted metagenomics) and, especially, for untargeted metagenomics. Moreover, DNA coming from the matrix (environmental DNA, eDNA) includes both the microbial DNA and the matrix (plant/animal DNA). Consequently, strategies should be used to reduce the amount of non-microbial DNA in the eDNA preparation or to select in the targeted metagenomics, microbial-specific target genomic regions or selective PCR primers (as for 16S rRNA, avoiding the amplification of plastid, hence plant-derived, 16S rRNA genes). Furthermore, depending on the level of sensitivity (i.e. the threshold of detection) the number of sequence reads per sample should be chosen in order to detect even low abundance taxa and to allow a good coverage of the microbial diversity in the sample. Finally, standardized procedures for sequence binning, operating taxonomic unit (OTU) attribution and assignment to microbial taxonomy must be selected after careful evaluation. This is especially critical for the database of taxonomic ribosomal references, as for instance Greengenes [34], RDP [35] or Silva [36], where different databases or different confidence thresholds may produce ambiguous microbial identifications.

However, from a food safety microbiology perspective, HTS will still require standardization of procedures for sample preparation, nucleic acid extraction, sequence data processing, etc. Moreover, HTS, tough their cost is decreased in the last years remain more expensive than Real-Time PCR in the case just a small number of targets has to be evaluated, and require more time from sample preparation to the bioinformatic data report.

3. Proteomics

In the last decade, we all witnessed a strong increase in the application of proteomics in the study, control, and validation of industrial processes of food products and in the assessment of food quality, safety, and traceability [14]. In particular, an enormous work has been done in the field of the meat, fish and dairy industry contributing for example to the identification of the specific biomarker for meat tenderness or traceability, fish flavour or to the evaluation of milk quality for the improvement of infant formulas and cheese making. In the case of proteomics applied to agriculture, a strong effort has been made in the assessment of the quality control of food product derived from transgenic crops, in the quality control processes of beer and wine industry and also in the characterization of the composition and quality of ready-to-eat vegetable products [37]. Another important issue that has been addressed by proteomics is food safety represented by the possible contamination by pathogenic microorganisms and microbial toxins, allergens, and toxic compounds. In this paragraph we will review the latest methodological approaches used in proteomics (gel-based and gel-free) such as comparative 2D electrophoresis, quantitative isotope labeling as well as the most recent and innovative label-free quantitative proteomics techniques, providing some examples of their application in food analysis (Figure 3).

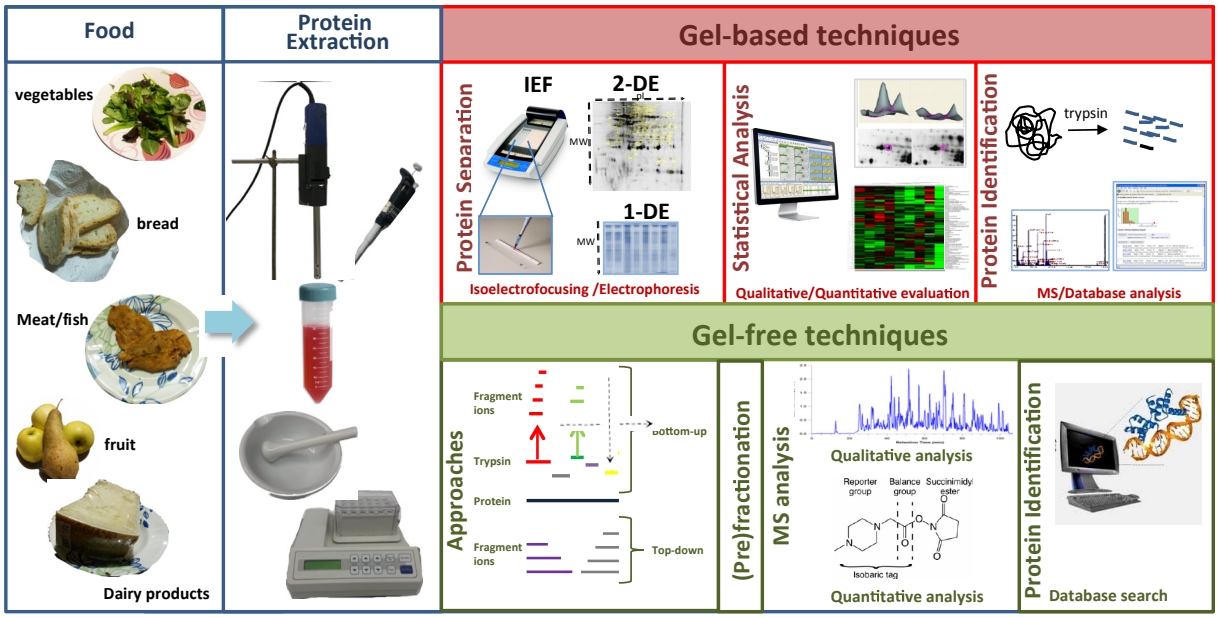

Fig. 3 Schematic representation of the proteomics analysis workflow using both gel-based and gel-free methodological approaches.

3.1 Gel-based approaches

The electrophoretic protein separation approach combined with MS analysis has become one of the most used methodologies in the food control, safety, and traceability. In fact, among gel-based techniques, two-dimensional gel electrophoresis (2-DE) has been widely used in the identification of quality markers of various foods, from meat and fish to vegetables, of biological or transgenic origin [38].

The major advantage of this technique is that 2-DE provides the highest protein-resolution capacity with a low-instrumentation cost. The typical workflow consists of the first phase of protein extraction from food sample (Figure 3). Then proteins are isolated by a bidimensional separation based on isoelectric focusing point and molecular weight on the SDS-polyacrylamide gel (PAGE). In the second phase after protein staining, the image analysis, using dedicated software, allows determining quantitative and qualitative variation, comparing the intensities of protein spots in different gels. In the third phase proteins from individually excised spots are enzymatically digested with trypsin, and the resulting peptides are analyzed by mass spectrometry to be finally identified by database searching [39].

The main limitations of this methodology are represented by the experimental variations that occur among gels. An advance in 2-DE is offered by the differential in gel electrophoresis technology (DIGE), in which the gel-to-gel variance is solved for comparative proteomics. In fact, in this technology, up to three samples can be differentially labeled and run on the same gel, increasing confidence in the detection and quantification of differences in protein abundance, taking advantage of the introduction of an internal standard that suppresses inter-gel variability [40]. There are only a few examples of the application of this technology to the study of food products, and these are related to the characterization of breed muscle proteome profiles [41], to investigate changes between GMOs and their counterpart non-GMOs [42], and very recently to the study of shelf-life storage process in fresh-cut and ready-to-eat vegetables [43].

Fish and prawn species authentication case.

Marine species in seafood products are often subjected to authentication not just for commercial fraud due to replacing quality materials with low-level ones, but also because they contain allergenic or toxic compounds dangerous for the health of consumers. Therefore, European legislation recommends seafood products labeling (Regulation (EU) No 1379/2013 of the European Parliament and of the Council of 11 December 2013 on the common organisation of the markets in fishery and aquaculture products).

2-DE proteomics approach thanks to sensitivity and possibility to be applied at a large-scale demonstrated to be a suitable analytical method to distinguish seafood species and to quantify their levels in seafood products.

Different 2-DE protein profiles of water-soluble proteins have been used to discriminate very closely related fish species [44]. In particular, studies based on 2-DE analysis revealed proteins such as triose phosphate isomerase isoforms, pyruvate kinase, troponin T, and beta-enolase as specific markers for distinguishing various species of tuna as Thunnus thynnus, Thunnus alalunga, Thunnus albacares, and Thunnus obesus [45]. A 2-DE approach has also revealed a different electrophoretic mobility of several spots together with a qualitative presence in farmed cod samples allowing to differentiate wild cods from farmed ones [46]. Moreover, few studies have been focused on crustaceans, known to be highly allergenic, to highlight differences among the closely related group of Decapoda shrimps and prawns. A proteomic gel-based approach successfully revealed different protein patterns in the most commercially relevant shrimp species. 2-DE profiles showed that the sarcoplasmic protein arginine kinase (AK) isoforms were differently modulated in six different species, proposing it as a biomarker for discrimination of Decapoda species also in mixed food products [47]. Moreover, 2DE represents also a useful method for food security purposes since it allows to select and quantify the levels of this protein, known to be highly allergenic.

3.2 Gel-free approaches

Despite the robustness of 2-DE techniques, gel-based proteomics suffers from intrinsic limitations, that prevent the separation of highly hydrophobic, extreme isoelectric point, or high MW proteins. Therefore, the scientific community has oriented, in recent years, in favour of complementary methods, globally known as mass spectrometry (MS)-based proteomics. This type of approach is rapidly emerging as a pivotal proteomic technology for the determination of food quality, authenticity, functionality, and safety.

It is characterized by a very large variety of analytical strategies and instrumentations whose detailed description is beyond the scope of this review. Nevertheless, all the different MS-based proteomic approaches share a common workflow, made up of three fundamental stages: 1) isolation and (pre)fractionation of protein sample; 2) qualitative and quantitative analysis by MS or MS/MS and 3) and assignment of MS or MS/MS spectra to peptides and proteins through database searching [48] (Figure 3).

Basically, two different workflows have been currently developed: bottom-up and top-down approaches. In the first, usually referred as shotgun proteomics, protein sample at stage 1 is enzymatically digested and the resulting complex peptide mixture is subjected to fractionation and analyses. Conversely, top-down proteomics is based on pre-fractionation, injection, and analyses of intact proteins. With respect to quantitative analysis, MS-based proteomics

offers at least three different approaches: label-based, label-free and targeted quantitation methods. We refer the readers to specific reviews for further information [49].

Targeted analyses offer the possibility to monitor and quantify specific proteins of interest (e.g. biomarkers). Multiple reaction monitoring (MRM) is becoming the central platform in for targeted analysis of protein/peptide abundances in complex matrices, food included. Depending on the type of instrumentation used, MRM assays can perform multiplexed analyses of hundreds of peptides in a single run [50].

The Milk case

Milk and dairy products have been extensively investigated by using proteomics [51]. Milk is characterized by a wide dynamic range, displaying high abundance proteins (e.g. caseins) and medium to low abundance whey proteins (e.g. ß-lactoglobulin, lactoferrin, immunoglobulins, glycoproteins, peptide hormones, and enzymes) [52]. First studies were addressed to the characterization of breast milk for its importance in newborn nutrition. The "classical" 2DE-MS proteomic approach led to the identification of 107 protein spots, corresponding to 39 gene products, in milk fat globule membrane proteins [53]. More recent works based on gel-free shotgun deeply penetrated into human milk proteome, identifying 268 gene products by using cutting-edge MS platform [54]. The evolution of high-throughput MS-technologies has paved the way to a more detailed characterization of breast milk, cow's milk, and dairy products. Milk transformation requires heating processes to guarantee its microbiological safety and a longer shelf-life. However, thermal treatments result in chemical modifications of milk proteins, affecting their nutritional, nutraceutical, biological and toxicological properties. Shotgun proteomic approach has been applied for protein structural analysis of glycation and glycoxidation in raw, pasteurized, UHT and powdered infant formula milk samples, identifying several protein targets in diverse heated samples [55]. MS-based technologies implementing MRM (Multiple Reaction Monitoring) scan function offer the possibility to detect and quantify specific biomarkers in complex food matrices. This strategy has been successfully applied for the detection of adulteration in buffalo mozzarella, by monitoring and quantifying the phosphorylated β-casein f33-48 peptide, identified as a specific proteotypic marker [56].

In conclusion, the rapid advances in proteomics, mainly represented by the use of gel-free approaches alongside with the more traditional gel-based methodologies, are providing valuable data such as quality/quantitative protein biomarkers that, in some cases, already found an application in the validation of industrial processes of food products and in the assessment of food quality, safety and traceability.

4. Transcriptomics

The study of a total set of transcripts in a given organism (namely transcriptome), at any given time and under any condition, allows to obtain meaningful insights into the functional elements of a genome and to elucidate molecular mechanisms underlying complex biological processes.

Comparative analysis of the transcriptome composition is a powerful tool to identify transient alterations in gene expression in response to genetic and/or environmental cues and in response to the general metabolic state of a given cell or tissue. This approach has been widely used to improve quality and quantity of food crops, as well as to characterize quality and safety of food (i.e. detection of transcripts involved in the accumulation of contaminants, bioactive molecules, nutrients, health effect molecules, etc.) [11].

High throughput transcriptomics became first possible with the development of the microarrays technique through the interrogation of thousands of gene targets by hybridization of RNA samples to oligonucleotide probes laying on miniaturized devices (Figure 4) [57].

In food crops, microarray-based approaches have been particularly useful to compare fluctuations in gene expression profiles in response to a wide range of conditions, like drought, high-salinity, cold, heat and disease [58]. The major drawback is that microarrays can detect only known gene sequences so they can't be used for the discovery of alterations of unknown genes. However, in the past few years, transcriptomics has expanded dramatically because of the advent of next generation sequencing (NGS) and genome-wide RNA sequencing (RNA-seq) (Figure 4) [59]. This latter is a more versatile technology to detect alterations of the transcriptome and is progressively overtaking the microarray-based approaches. It can easily provide, in fact, high-resolution gene expression, detection of lower abundant transcripts, point mutations, alternative splicing as well as new coding and noncoding RNA transcripts, including small regulatory RNAs (sRNAs) and antisense transcripts [60].

Nowadays, the massive sequencing data available on the genome structure of thousands of species has allowed performing transcriptomic assays based on genome-wide microarrays and RNAseq on many crop and animal species to investigate gene functions involved in the accumulation of compounds of interest for human health and for the overall food quality. The intrinsic nature of the RNA-based investigations requires fresh food substrates with minimal processing. RNA molecules are very delicate and undergo a quick degradation as cell structures lose their metabolic activity and are dismantled during the preparation of certain food products which require high temperatures and complex manipulation of the basic ingredients. In such foods, transcriptomic investigations become in fact more and more challenging and are conveniently replaced by other biochemical assays to detect proteins or metabolites.

Nevertheless, transcriptomics of fresh unprocessed food products has proven to be very effective as a diagnostic tool to infer about many attributes of foods through detection of altered gene expression. In this context, an example of a recent transcriptomic study elucidate biosynthetic processes underlying the regulation of bitter taste in chicory, which is a crucial attribute of quality in terms of the product acceptability [61]. It's known that sesquiterpene lactones (STLs), secondary metabolites typical of Asteraceae spp., confer the bitterness of chicory, in addition to other nutritional, allergenic and healthy properties. Analysis of differentially expressed genes in two stem-chicory "Catalogna" landraces, characterized by different bitterness scores, identified several STL genes strictly associated to bitterness which promise to be useful markers for food quality.

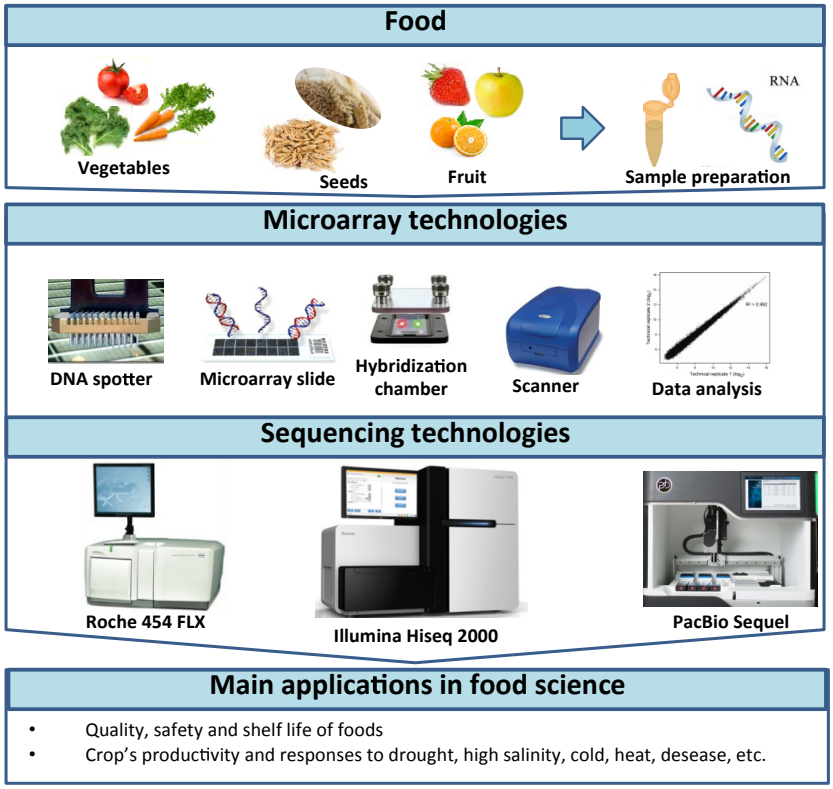

Fig. 4 Overview of food transcriptomics.

Gene expression analysis is also useful to enhance food quality and improve the shelf life of products by investigating molecular mechanisms which take place during storage and post-harvest treatments. Mellidou and colleagues explored changes of the apple transcriptome associated with a flesh browning disorder related to controlled atmosphere storage [62]. The identified candidate genes associated with internal browning in a tissue-specific manner represent markers of the browning incidence in apples during storage in the controlled atmosphere and could be useful to improve the postharvest life of apple, as well [62].

A further application of transcriptomics is to detect food contaminants by fingerprinting methodologies [12]. Living cells display wide adaptive plasticity by responding with characteristic expression changes to the toxic agents possibly present in foods. Two human cell lines have been used to explore the impact of microarray-based transcriptomic analyses for detection of different contaminants. Interestingly, both cell lines yielded characteristic expression profiles following the exposure to chemicals that are often found in food products. In this context, the transcriptomics approach can expand the range of contaminants that can be detected in a single experiment and increase the specificity of the cellular response [12].

Transcriptomic techniques have demonstrated their impressive analytical potential for gene expression studies in the context of food science. However, the applicability of RNA-based assays in food science is still in its infancy and it is not fully exploited yet.

Though RNA-Seq has quickly superseded microarrays in many gene expression studies, it still remains evolving and several technical and bioinformatics challenges need to be overcome to realize the full potential of this technique in food science. Given the evolution path of RNA-Seq technology, high-end instruments with higher sequencing throughput able to provide longer and accurate reads can be expected in the very near future. For instance, a new generation of sequencers, based on single- molecule and single- cell sequencing, is rapidly emerging [63]. In addition to their capability for sequencing RNA directly, novel technologies can also sequence molecules in real-time, decreasing the time of analysis and allowing higher sensitivity. The improved analytical performances made available

by the new sequencing technologies can provide more detailed information about the transcriptional regulation in real samples boosting the applicability of transcriptomics in food science.

5. Metabolomics

The study of the entire metabolite composition of a living cell, organism or a particular system has been defined as metabolome, and metabolomics allows the determination of the metabolic composition of different matrices. Since the metabolome represents the final biochemical results of gene expression and environmental conditions, metabolomic approaches have emerged as reliable and powerful tools able to extend the knowledge on food biochemical composition and to investigate the quality and safety of different food matrices [64]. Metabolomics basically comprises targeted and untargeted approaches [65]. While targeted metabolomics aims to selectively profile several metabolites, and usually requires a specific extraction of metabolites, the untargeted approach is used to extrapolate significant differences in a comparative manner, allowing to apply a metabolic fingerprinting or to identify new compounds as biomarkers for food safety and quality [66].

Like other "omic" disciplines, metabolomics involves instrumental analysis, which is optimized to obtain the most comprehensive metabolic data set, and data analysis workflow, which consists of the bioinformatics/statistic tools used to process high-throughput data sets. Key steps for obtaining such metabolic data set are i) extraction, ii) separation, and iii) detection of metabolites (Figure 5).

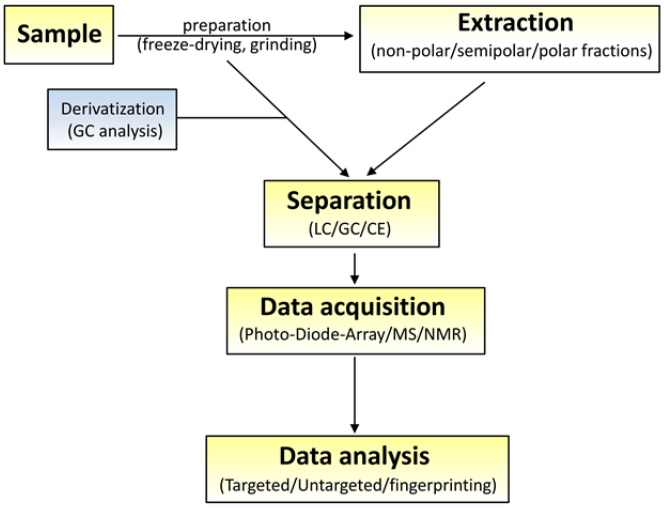

Fig. 5 Pipeline for food metabolomics.

Different protocols have been established for the extraction of specific classes of metabolites or for global profile studies [66]. In the targeted approach metabolites extraction often includes sample clean-up step using Solid Phase Extraction (SPE) or Solid Phase Micro-extraction (SPME), while to obtain a global metabolite profile different extraction protocols are used in order to increase the metabolites diversity. The separation step is in general accomplished by liquid chromatography (LC), mainly with its high performance (HPLC), ultra-performance (UPLC) or ultra-high performance (UHPLC) forms, gas chromatography (GC) and capillary electrophoresis (CE). However, in some cases, direct infusion (DI) is used to obtain a metabolic profile. Numerous techniques have been developed for metabolites detection, such as Fourier transform infra-red spectroscopy (FTIR) [15], nuclear magnetic resonance (NMR) [16] and mass spectrometry (MS) [17] although in food metabolomics MS and NMR have been used the most [65]. While NMR gives high-resolution spectra, allowing to identify the exact structure of metabolites, in MS the mass analyzers used ranged from high resolution MS (HRMS) [17], such as Fourier transform ion cyclotron resonance (FTICR), Orbitrap and Time of Flight (ToF), to low-resolution MS like quadrupole (Q), ion-trap (IT), ion-mobility spectrometry (IMS) and hybrid systems. Clearly, HRMS is preferred because of its high mass accuracy and sensitivity [17]. The acquired data are then analyzed using different statistical and chemometric approaches. Many metabolite databases have been developed, helping the identification of unknown metabolites present in food matrices. Due to the advancements in analytical instruments in the last decade, it is now possible to analyze thousands of metabolites from a food sample in a single analysis. The sample preparation and data handling processes have also been improved tremendously, making it easier for scientists to analyze samples in a short time.

In the frame of food quality, one of the major challenges of metabolomics is to develop fast, reproducible and cost-effective analytical methods able to assess food quality, with the ultimate purpose to protect and improve the health of consumers, satisfying their expectations. Although the quality of raw materials is basically due to intrinsic traits of the

sample, the final products of food processing derive from raw ingredients combined and transformed to produce marketable products, thus final food-quality is given by multiple processing factors, as well as by storage and packaging conditions. A large number of MS-based and HRMS-based approaches have been applied in order to improve food quality, developing robust and fast methods able to analyse the wide range for nowadays known health-related metabolites, such as phytochemicals (carotenoids, polyphenols, see for example [67]), vitamins (provitamin A and vitamin C) [68] fatty acids (omega-3 and omega-6 FA) [69] and minerals (calcium, potassium and magnesium) [70]. Recent MS-based approaches have been used, for instance, to simultaneously quantify several abundant bioactive compounds such as carotenoids, tocopherols and free and esterified sterols, in important food product such as canola, olive, and sunflower oils, by using a target HPLC-DAD-MS/MS strategy, a simple extraction procedure and 30 minutes total runtime [71].

Since new MS technologies are able to produce large dataset with thousands of potentially quality-related compounds, metabolomic approaches have been used in combination with multivariate analysis, such as PCA (Principal Component Analysis), in order to extrapolate significant information, as has been described, for instance, for selected specific metabolite patterns discriminating fruit from several commercially grown cultivars, demonstrating that specific metabolites correlate directly with quality traits [72]. Since flavour is one of the most important criteria influencing consumer acceptance and quality of foods, an increasing number of GC-MS based approaches have been extended to discover metabolites that significantly contribute to these traits. GC-MS based approach has been recently used to identify 33 compounds correlating with consumer liking, and 37 that significantly correlated with flavor intensity. These compound have been further used to design a genome-wide association study (GWAS), identifying candidate loci capable of altering 15 of the chemicals contributing to consumer liking and an additional 6 chemicals that contribute to overall flavour intensity of tomatoes [73].

In addition, metabolomics represents a powerful tool for the determination of food contaminants like toxins (mycotoxins, mainly found in cereals, and algal toxins that contaminate marine-related foods) [74], chemicals (including pesticides, pollutants, antibiotics and growth-promoting agents) [75], spoilage microorganism (*Pseudomonas spp*., *Acinetobacter spp*., *Botrytis spp*.) and pathogens (*Escherichia coli* strains, *Salmonella spp*., *Shigella spp*., *Listeria monocytogenes*, *Campylobacter jejuni* and viruses, such as norovirus and rotavirus) [76] or genetic modified (GM) ingredients [77].

Both targeted and non-targeted metabolomics have been used for the detection and identification of such contaminants. While targeted analysis by using NMR or MS are suitable for the study of already-known contaminants, the untargeted approach is indicated to identify novel compounds that can be used as biomarkers for the identification of illegal practices in food production and to monitor the integrity and safety of food products.

The method of choice for toxins detection is represented by LC-HRMS or LC-MS/MS. Simultaneous detection of several toxins has been successfully reported by the use of high-resolution analyzer [78]. LC-HRMS protocols were also established in the case of chemicals identification such as pesticides, antibiotics and growth-promoting agents [75], and comparative metabolic profiles of GM and non-GM crops have been performed using targeted and untargeted LC-HRMS and NMR [80]. Metabolic fingerprinting using GC-MS have been efficiently established for the determination of spoilage or pathogenic microorganisms that produce volatile organic compounds (VOC) [80] [81]. Other primary metabolites such as sugars and amino acids were characterized as biomarkers for early food contamination by pathogens [81]. GC-MS also remain the best technique for the determination of environmental pollutants in food such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons or polybrominated diphenyl ethers [82].

6. The need for data integration: system biology and foodomics

The development of high-throughput technologies has led to the generation of large “omics” dataset that allowed the identification of novel biomarkers for food safety and quality. However, considering the dynamics of biological systems, multi-level integration studies can result particularly useful in this context. Integration of genomics, transcriptomics, proteomics and especially metabolomics, to study the interactions among molecular components and their changes induced by perturbations in a biological system is an emerging discipline named systems biology [83]. Basically, two approaches, the data-driven and the model-driven, are used to study biological systems [83] [84]. The first approach is useful to identify novel components and their interactions from large-scale omics datasets in order to define new metabolic functions. In general, molecular interactions are analyzed by correlation networks that can be visualized as graphs in which the components are described by nodes and the interactions are shown as edges [85]. The model-driven approach can be then used to interpret the behavior of the system. In general, a mathematical model of the biological system is constructed and matched against global observations in an iterative manner, in order to obtain a model that reflects biological reality [85].

The development of systems biology is beneficial to the study of the impact of food compositions and ingredients on human health [20]. The traditional food research is moving from classical methodological methods to new analytical approaches aiming at integrating biological data with bio-informatics tools. The new omics technologies combined with system biology can lead food research into a new era, the foodomics, which was defined in 2009 as a discipline that

studies the food and nutrition domains through the application and integration of advanced omics technologies to improve consumer's well-being, health, and knowledge [86]. Currently, in the so-called globalization, in which the movement of food and related raw-materials worldwide requires the ensuring of safety, quality, and traceability of products, the development, validation, and implementation of rapid, sensitive, and accurate methods for assessment of food safety are needed [87]. Foodomics involves the use of genomics, proteomics, transcriptomics, and metabolomics, including nutrigenomics, for compound profiling, authenticity, and/or biomarker detection related to food quality and safety [2]. It has already been applied in different fields of science and food technology and nutrition; i.e., evaluation of food safety and quality, the study of the effects on human health of different bioactive food components, the individuation and quantification of dietary biomarkers related to different health conditions, or the assessment of biological responses to different nutritional patterns. An especially complex challenge in the combination of Foodomics and system biology is the possibility of connecting food components, foods, diet, the individual, including food impact on health and illness, by considering the food domain as a whole with the nutrition domain. Only in this way, it will possible to account for food products tailored to promote human health and well-being.

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