Chapter 5
Mass Spectrometry Applications

Carla Soler, Josep Rubert, and Jordi Mañes

5.1 Proteomics and Mass Spectrometry

Over the last decades, food safety and quality control in all production processes have attracted a great deal of attention. Food safety itself might be implicit in the broader concept of food security, defined as a situation in which all people at all times have physical and economic access to sufficient, safe, and nutritious food to meet their dietary needs and live an active healthy life (World Food 1996).

In recent years, food safety is an increasingly broad concept that encompasses mainly three main areas: (1) food quality (food composition), (2) traceability (food origin), and (3) food safety per se (absence of allergens, pathogens, or other contaminants) (D'Alessandro and Zolla 2012). Early attempts to individuate quality standards through biomolecules mainly relied on rough biochemical parameters, such as product composition (e.g., through labeling of percentages of lipids, carbohydrates, proteins, and vitamins). Recent advancements in the fields of biochemistry and molecular biology allowed us to look for actual biomarkers to be potentially exploited as indicators of product quality and traceability (Cairns 2011). In this frame, proteomics have already contributed to accumulating a relevant body of knowledge that might result not only in improving food safety through enhancing selection of food quality and traceability markers but also in providing the end-user consumer with a unique tool to make a fully aware alimentary choice (D'Alessandro and Zolla 2012)

C. Soler (✉) • J. Rubert • J. Mañes
Departament de Medicina Preventiva i Salut Pública, Universitat de València,
Av. Vicent Andrès Estellès, Burjassot 46100, Spain
e-mail: carla.soler@uv.es

F. Toldrá and L.M.L. Nollet (eds.), *Proteomics in Foods: Principles and Applications*,
Food Microbiology and Food Safety 2, DOI 10.1007/978-1-4614-5626-1_5,

The history of proteomics dates back to the discovery of two-dimensional gels in the 1970s, which provided the first feasible way of displaying hundreds or thousands of proteins on a single gel (Klose 1975; O'Farrell 1975). Identification of the spots separated on these gels remained laborious and was limited to the most abundant proteins until the 1990s, when biological mass spectrometry had developed into a sufficiently sensitive and robust technique.

Mass spectrometry was restricted for a long time to small and thermostable compounds because of the lack of effective techniques to softly ionize and transfer the ionized molecules from the condensed phase into the gas phase without excessive fragmentation. The development in the late 1980s of two techniques for the routine and general formation of molecular ions of intact biomolecules (electrospray ionization, ESI) (Fenn et al. 1989) and matrix-assisted laser desorption/ionization (MALDI) (Karas and Hillenkamp 1988) dramatically changed this situation and made polypeptides accessible to mass spectrometry analysis.

In recent years, mass spectrometry has evolved into an indispensable tool for proteomics research. Coupled with an isolation technique to purify a specific protein complex of interest, MS can rapidly and reliably identify the components of complexes. For MS experiments, consideration should be given to the type of instrumentation, fragmentation method, and analysis strategy best suitable to an individual sample (Aerbersold and Goodlett 2001).

The strategy followed to characterize protein complex composition using MS has been well schematized by Gingras et al. (2005): a protein complex to be analyzed is first purified using an appropriate approach. The purified protein may then be separated using techniques such as SDS-PAGE, isoelectric focusing, or various two-dimensional separation methods. Individual proteins may then be visualized by staining, and recovered for analysis. Isolated protein can be proteolytically digested (most often using trypsin) to generate a mixture of peptides that can be identified by MS. The proteins present in the sample are deduced by recombining the identified peptides, using suitable informatics tools.

Currently, the uses of MS in proteomics are in three major areas. MS is the preferred technique in the field of biotechnology for characterization and quality control of recombinant proteins and other macromolecules. It is also commonly used for protein identification, either in classical biochemical projects or in large-scale proteomics ones. Finally, since MS measures the molecular weight of a protein, it is the method of choice for the detection and characterization of post-translational modifications and potentially can identify any covalent modification that alters the mass protein (Mann et al. 2001).

Different strategies can be followed to carry out a proteomic study including "top-down proteomics" (MS is carried out in the form of whole-protein analysis) and "bottom-up proteomics" (protein identification is carried out by the analysis of enzymatically or chemically produced peptides) (Han et al. 2008). In the first one, MS analysis can provide accurate molecular mass measurement of intact proteins as large as 100 kDa or more. Highly accurate protein mass measurements generally are of limited utility, because they often are not sufficiently sensitive and because net mass often is insufficient for unambiguous protein identification.

In the second one, MS analysis can provide accurate mass measurements of peptides from proteolytic digests. In contrast to whole protein mass measurements, peptide mass measurements can be done with higher sensitivity and mass accuracy (Liebler and Yates 2002).

There are four basic types of mass analyzer currently used in proteomic research; ion trap, time-of-flight, quadrupole, and Fourier transform analyzers. They are very different in design and performance, each with its own strength and weakness. These analyzers can be standalone or in some cases put together in tandem to take advantage of the strengths of each (Aebersold and Mann 2003).

The aim of this chapter is to review the major types of MS instruments, both currently in use and under development, and to discuss strategies for the analysis of intact proteins or for the analysis of peptides obtained after enzymatic degradation of the protein. Finally, application of mass spectrometry to three areas related to food safety such as the detection of micro-organisms, the determination of modifications induced by food processing, and the identification of protein components responsible for food allergy and intolerance are summarized.

However, it is important to keep in mind that proteomics still remain a multifaceted, rapidly developing, and open-ended endeavor. Although they have enjoyed tremendous recent success, proteomics still face significant technical challenges; each breakthrough that either allows a new type of measurement or improves the quality of data made by traditional types of measurements expands the range of potential application of MS to molecular and cellular biology. Indeed, this field is already too extensive for a comprehensive single review; thus it has been impossible to include all the achievements in MS-based proteomics.

5.2 Ionization Techniques

Ionization techniques are critical to convert molecules into ions that can then be manipulated within electric or magnetic fields. The challenge with biological molecules such as peptides and proteins is to convert polar molecules into gas-phase ions without degradation or fragmentation (Yates 2004). The development of ESI and MALDI solved this difficult problem of generating ions from large nonvolatile analytes such as protein and peptides without significant analyte fragmentation.

ESI gained immediate popularity because of the ease with which it could be interfaced with popular chromatographic and electrophoretic liquid-phase separation techniques and quickly supplanted fast atom bombardment as the ionization method of choice for protein and peptide samples dissolved in a liquid phase. This technique creates ions by spraying an electrically generated fine mist of ions into the inlet of a mass spectrometer at atmospheric pressure (Fenn et al. 1989). Steady advances in the application of ESI to the analysis of peptides and proteins have been made. The most notable improvements have come from reduction in the flowrate of

the liquid used to create the electrospray and thus more efficiently create ions (Emmet and Caprioli 1994; Smith et al. 1990). Furthermore, due to the propensity of ESI to produce multiple charged analytes, mass analyzers with limited *m/z* range could be used to detect analytes with masses exceeding the nominal *m/z* range of instruments (Aerbersold and Goodlett 2001).

For different but no less compelling reasons, MALDI also rapidly gained popularity. In this technique, a pulsed laser is used to deposit energy into the matrix, causing rapid thermal heating of the molecules to desorb molecules and ions into the gas phase. Because a pulsed laser is used, this ionization technique produces ions in packets rather than a continuous beam and thus requires a mass spectrometer that can either measure a complete mass spectrum without scanning a mass range or trap of all the ions for a subsequent mass analysis (Yates 2004).

MALDI mass spectra are simple to interpret due to the propensity of the method to generate predominantly singly charged ions. The method is relatively resistant to interference with matrices commonly used in protein chemistry (Aerbersold and Goodlett 2001). However, preparation of reliable sample spots onto the MALDI target demands a homogeneously applied peptide sample at the region upon which the laser shots will take place. Several factors affect the efficacious preparation of a MALDI target. These factors include the accurate application of matrix (pipette tip must not excessively touch the steel target but yet apply the full liquid volume), sample, and recrystallization solutions onto the steel plate spots, and the use of freshly prepared matrix and recrystallization solutions (Garbis et al. 2005).

5.3 Mass Spectrometers for Protein and Peptide Analysis

MS has played a pivotal role in proteomics research, with mass analyzers being central to the technology, and has proved to be a powerful analytical technique for both protein and peptide analysis (Chen 2008). Tandem MS (MS/MS) consists of either the fragmentation of a selected precursor peptide ion to generate specific fragment ions for sequence elucidation (data-dependent acquisition), or uncoupled acquisitions of intact and fragment masses with retrospective reconstitution of the parent–daughter ion context (data-independent acquisition) (Sénéchal and Kussmann 2011). By using tandem MS, a complete sequence of the peptides of interest is often achieved. To identify these peptides, spectra are scanned against protein-sequence databases using search algorithms (Lane 2005).

The most popular analyzers in proteomics are ion traps, triple quadrupoles, time-of-flight tubes, orbitrap, and Fourier transform ion cyclotron resonance, with their specific advantages: high sensitivity and multiple-stage fragmentation for ion traps, high selectivity for triple-quadrupoles, high sensitivity and speed for time-of-flight.

Figure 5.1 depicts the principal ionization sources and principal mass spectrometers used in proteome research.

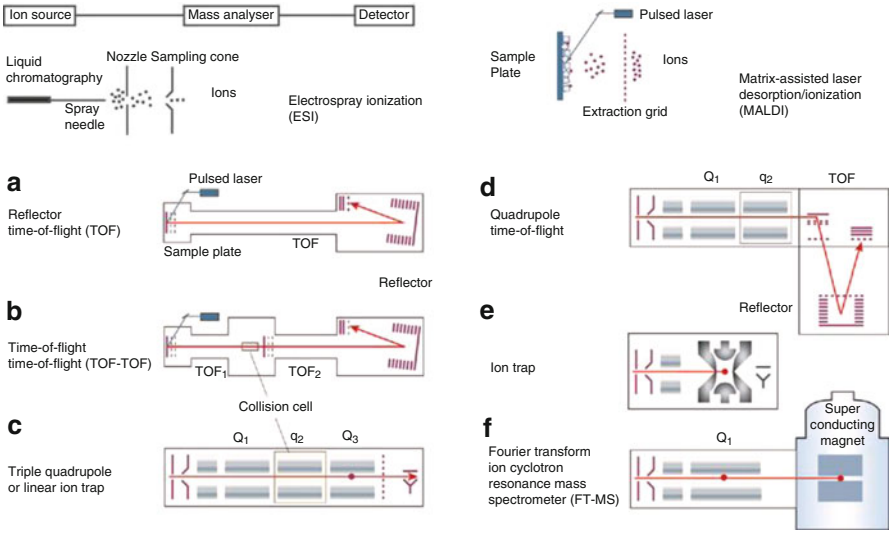

Fig. 5.1 The *left* and *right upper* panels depict the ionization and sample introduction process in electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). The different instrumental configurations (**a**–**f**) are shown with their typical ion source (Reproduced from Aerbersold and Mann (2003) with permission from Nature Publishing Group)

5.3.1 Time-of-Flight (TOF) Mass Analyzer

In theory the time-of-flight mass spectrometer is the simplest mass analyzer. Mass-to-charge (*m/z*) ratios are determined from the flight times of analyte ions traveling through a field-free region or a flight tube. At a constant accelerating voltage, the flight time for an ion is proportional to the square of the *m/z* ratio. The lighter ions (with lower *m/z* values) have a higher velocity and are recorded on a detector earlier than the heavier ones (higher *m/z* values), thus producing the TOF spectrum as a function of time (Shen and Noon 2004). The biggest drawback to TOF analyzers is their inability to perform true MS/MS. However, in 1993 the tandem time-of-flight (TOF/TOF) mass spectrometer was introduced (Cornish and Cotter 1993a, b)with two reflectron mass analyzers separated by a mass-selector gate and collision region into which a collision gas was pulsed once each mass recording cycle. These TOF/TOF instruments incorporate a collision cell between two TOF sections. Ions of one *m/z* ratio are selected in the first TOF section, fragmented in the collision cell, and the fragments are separated in the second TOF section (Guerrera and Kleiner 2005)

The scanning mass analyzers such as TOF are usually interfaced with MALDI to perform pulsed analysis. In fact MALDI-TOF or MALDI-TOF/TOF are relatively simple and robust to operate, have good mass accuracy, and high resolution and sensitivity. They are widely used in proteomics to identify proteins from sim-

ple mixtures by a process called peptide mass fingerprinting. In this approach, proteins of interest are digested with a sequence-specific enzyme such as trypsin and the generated peptides are analyzed by MS. The determined masses, usually in the range of 800–3,000 *m/z*, are then compared against a database comprising peptide masses from a virtual digest of all proteins from a given organism with the same sequence-specific protease (Cottrell 1994).

5.3.1.1 Drawbacks

The major drawbacks of this method are that (1) hydrophobic proteins such as membrane proteins, very basic proteins, and low abundant proteins are not well represented on the gels and (2) the procedure has low throughput and is difficult to standardize and automate.

A limitation of MALDI-TOF is the identification of low molecular mass proteins, which deliver few peptides, and the identification is often based on a low number of matches (Fountoulakis et al. 1998). In that case, it should be confirmed that the matching peptides are simultaneously the major peptides, and in general, identification of small proteins by MALDI-TOF-MS is not efficient, and the application of MS/MS technologies such as TOF-TOF, Qq-TOF, or LC–MS may be more advantageous.

Moreover, the MALDI-TOF-based protein identification approach cannot identify multiple components of a mixture. In most cases, in 2-D gels, the major component of a protein mixture is identified by MALDI-TOF from one spot. A spot often contains more than one protein and two or more additional proteins can be identified from the same spot as well, but they are usually highly homologous, and the software cannot distinguish between the identification hits. If experimentally derived peptide masses for a given mass accuracy tolerance correspond to more than one protein, the resulting search score becomes low (Westermeier and Marouga 2005). This problem is further aggravated when the number of experimentally derived peptides is small and the intensity of the peptide signal is weak. Additional proteins can be identified from spots by tandem mass spectrometry if a sufficient number of peptides have been analyzed in the MS/MS mode.

In most analyses, only a subset of the predicted masses is actually observed. Such a limitation is the result of multiple contributing factors including loss of peptides during the sample handling process, as described before, ion suppression effects due to the presence of trifluoroacetic acid, surfactant residues and salts, the selective ionization of certain peptide species over others depending on their amino acid sequence, which typically exhibit poor ionization potential, and small peptide masses that are beyond the optimum mass range of the MALDI-TOF instrument.

5.3.2 Ion Trap

In the IT analyzers ions are trapped and can therefore be accumulated over time in a physical device. These ions are then subjected to an additional electric field, which

ejects one ion species after another from the trap, and are detected, to produce a mass spectrum. The IT technology is characterized by MS/MS capabilities with unmatched sensibility and fast data acquisition. Used in conjunction with data-dependent acquisition, IT technology allows high-throughput (Domon and Aaebersold 2006).

Many proteomic studies use linear ion trap mass spectrometers to analyze complex peptide mixtures derived from proteolytic digestion of protein samples via online microcapillary liquid chromatography (μLC) and tandem mass spectrometry (MS/MS). Compared to earlier ion trap instruments, the trapping of ions in an axial fashion in the linear ion trap provides significant improvements, increasing ion capacity, scan rate, and improved detection and trapping efficiency. Consequently, these instruments identify proteins by MS/MS from complex biological mixtures with unsurpassed sensitivity, which, combined with their low maintenance, ease of use, and relatively low cost, have made linear ion traps the instrument of choice for a wide variety of proteomic studies. (Griffin et al. 2007).

5.3.2.1 Drawbacks

When only low-resolution spectrometers and low-energy fragmentation techniques are available the MS/MS-based identification of nontryptic peptides becomes a very hard task. In this case even more refined programs developed for peptide identification, such as those also exploiting relative abundances produced at different levels of the procedure (Eng et al. 1994; Narasimhan et al. 2005), may not be able to provide a unique reliable result.

Another limitation of linear ion traps, commonly known as the "one-third rule," arises from decreased stability of fragment ions with *m/z* values less than 30% of the *m/z* for the precursor peptide selected for fragmentation by collision-induced dissociation (CID) during automated MS/MS analysis. For example, fragment ions from an ion at *m/z* 1,500 will not be detected below *m/z* 500, a significant limitation for the de novo sequencing of peptides.

5.3.3 Triple Quadrupole

ESI-MS with quadrupole instruments has been demonstrated to be a powerful tool for mass determination of food proteins and for detection of protein modifications induced by processing (Careri et al. 2002). The quadrupole is a mass filter consisting of four rods to which an oscillating electric field is applied and which lets only a certain mass pass through. The triple quadrupole is composed of two of these quadrupoles; these are separated by a somewhat different quadrupole which serves as a collision cell leading to peptide ion fragmentation (Mann et al. 2001).

One of the first people to exploit the triple quadrupole for protein sequencing by tandem mass spectrometry was Don Hunt (Hunt et al. 1986). Hunt championed the use of low-resolution parent ion selection and daughter ion detection to maximize sensitivity, and chemical modification (peptide methylation and acetylation) to aid the determination of ion series for interpretation.

Triple quadrupoles were the original instruments used for tandem MS in proteomics studies. The accuracy of quadrupole mass analyzers allows selection of specific peptide ions and analysis of fragment ions from MS-MS to within at least ±0.5 amu of their true *m/z* values. This degree of mass accuracy is sufficient to allow direct interpretation of amino acid sequences from peptide MS-MS data obtained with this mass spectrometer (Liebler and Yates 2002).

5.3.3.1 Drawbacks

To apply a "top-down" approach, selected reaction monitoring (SRM) using triple-quadrupole mass spectrometers has been developed (Ji et al. 2003); in this mode, the "parent" ion and its principal obtained fragments are selected. Although SRM detection provides excellent selectivity and duty cycle, it also has some inherent disadvantages in fragmentation-dependent MS detection. As the molecular weight of a protein increases, the fragmentation efficiency of the protein through the SRM process often decreases. Even when the protein is fragmented, the charges are more evenly distributed into many fragments rather than concentrated onto a few major fragments as with small molecules. Furthermore, the electrospray ion source commonly used on triple-quadrupole MS tends to ionize a protein into different charge states. SRM can only detect the one particular fragment transition from a precursor ion at a specific mass-to-charge ratio, whereas the majority of the ions in all the other charge states cannot be utilized at the same moment (Ruan et al. 2011).

5.3.4 Orbitrap

As its name suggests, orbitrap is an ion trap. But it is not a conventional ion trap; there is neither RF (radio frequency) nor a magnet to hold ions inside. Instead, moving ions are trapped in an electrostatic field (Makarov 2000). The electrostatic attraction towards the central electrode is compensated by a centrifugal force that arises from the initial tangential velocity of ions, very much like a satellite in orbit. The electrostatic field that ions experience inside the orbitrap forces them to move in complex spiral patterns. The axial component of these oscillations is independent of initial energy, angles, and positions, and can be detected as an image current on the two halves of an electrode encapsulating the orbitrap. A Fourier transform is employed to obtain oscillation frequencies for ions with different masses, resulting in an accurate reading of their *m/z* (Scigelova and Makarov 2006).

The orbitrap is a high mass accuracy detector. High and accurate mass measurement has led to new proteomic methodologies; the better characterization of the intact protein state and on the other hand the use of accurate mass tags for digested proteins minimizes the need for time-consuming MS/MS-based peptide identification and can, in principle, improve throughput. Protein quantification is also improved by accurate mass analysis (Marshall and Hendrickson 2008). In fact, the literature supports claims of routine mass measurement accuracies of less than 2 ppm for the analyses of complex peptide mixtures (Yates et al. 2006) and even intact proteins (Macek et al. 2006).

5.3.5 New Types of Mass Spectrometers: The Hybrid Instruments

The general goal in the design of a hybrid instrument is to combine different performance characteristics offered by various types of analyzers into one mass spectrometer. These performance characteristics may include mass resolving power, the ion kinetic energy for collision-induced dissociation, and speed of analysis (Glish and Burinsky 2008). Table 5.1 summarizes the performance parameters for the various analyzers.

For example, as commented before, the biggest drawback to the TOF analyzers is their inability to perform true MS-MS, however, developed TOF-TOF instruments have overcome this problem. These TOF-TOF instruments incorporate a collision cell between two TOF sections; ions of one *m/z* ratio are selected in the first TOF section, fragmented in the collision cell, and the fragments are separated in the second TOF section (Guerrera and Kleiner 2005).

The combination of triple-quadrupole and ion-trap mass spectrometer functionality inherent in the QTRAP™ instruments means that highly selective scan modes for analyte identification can be combined with very sensitive scans for structural identification on a chromatographic timescale (Hager 2004).

The unique scanning capabilities of a hybrid linear ion trap (Q TRAP) mass spectrometer are described with an emphasis on proteomics applications. The combination of the very selective triple-quadrupole-based tandem mass spectrometry scans with the very sensitive ion trap product ion scans allows rapid identification of peptides at low concentrations derived from post-translationally modified proteins on chromatographic timescales. The Q TRAP instrument also offers the opportunity to conduct a variety of ion processing steps prior to performing a mass scan. For example, the enhancement of the multiple-charge ion contents of the ion trap can be performed resulting in a survey mass spectrum dominated by double- and triple-charge peptides. This facilitates the identification of relevant biological species in both separated and unseparated peptide mixtures for further MS/MS experiments (Le Blanc et al. 2003).

In addition, highly resolved and accurate orbitrap measurements for the parent ions are complemented with tandem mass spectra detected in a linear ion trap with high speed and sensitivity. LTQ orbitrap hybrid instruments offer the additional

Table 5.1 Comparison of mass analyzers with respect to key performance attributes (Reproduced from Glish and Burinsky (2008) with permission from Elsevier)

Analyzer type	Kinetic energy	Resolving power		Mass accuracy		Measurement time[a] (s)
		MS^1	MS^2	MS^1	MS^2	
Magnetic (B) sector	High	Medium	Medium	ppt	ppt	10^{-5}
Electric (E) sector	High	na[b]	Low	na[b]	ppt	10^{-5}
Electric and magnetic sector[c]	High	High	Medium	ppm	ppt	10^{-5}
Time-of-flight (TOF)	High	Low	Medium[d]	ppm	ppm	10^{-5}
Quadrupole mass filter (Q)	Low	Low	Low	ppt	ppt	10^{-4}
Quadrupole ion trap (QIT)	Low	Low	Low	ppt	ppt	10^{-2}
FTICR	Low	High	High	ppm	ppm	10^{-1}
Orbitrap	High	na[b]	High	na[b]	ppm	10^{-1}

[a]The time, after activation, for the ions to react prior to the second stage of analysis

[b]na: not applicable; this analyzer has not been used for the first stage of an MS/MS experiment. There is currently no mode of operation in which a parent ion can be mass selected for a subsequent stage of analysis

[c]When electric and magnetic sectors are used as a double focusing mass spectrometer for one stage of analysis, either prior to (MS-1) the reaction, or subsequent to (MS-2) the reaction

[d]The resolving power is medium if the TOF ion axis is orthogonal to the first stage of MS; if the axes are coaxial, the resolving power is low

option of transferring the product ions (created in the linear trap) to the orbitrap mass analyzer for analysis with high resolution and high mass accuracy. An orbitrap mass analyzer is the most recent addition to the set of tools that can be applied to identification, characterization, and quantitation of components in biological systems. With its ability to deliver low-ppm mass accuracy and extremely high resolution, all within a timescale compatible with nano-LC separations, the orbitrap has become an instrument of choice for many proteomics applications since its commercial introduction in 2005 (Scigelova and Makarov 2006).

More recently the Q-Exactive instrument has appeared, which features high ion currents because of an S-lens, and fast high-energy collision-induced dissociation peptide fragmentation because of parallel filling and detection modes. In this mass analyzer the orbitrap is coupled with a quadrupole and the image current from the detector is processed by an "enhanced Fourier transformation" algorithm, doubling mass spectrometric resolution. This is demonstrated in a multiplexed single ion monitoring mode, in which the quadrupole rapidly switches among different narrow mass ranges that are analyzed in a single composite MS spectrum. Similarly, the quadrupole allows fragmentation of different precursor masses in rapid succession, followed by joint analysis of the higher energy collisional dissociation fragment ions in the orbitrap analyzer.

More than 2,500 proteins can be identified in standard 90-min gradients of tryptic digests of mammalian cell lysate, a significant improvement over previous orbitrap mass spectrometers. Furthermore, the quadrupole orbitrap analyzer combination enables multiplexed operation at the MS and tandem MS levels. The high performance together with the ability to perform complex multiplexed scan modes makes the Q Exactive an exciting new instrument for proteomics (Michalski et al. 2011).

5.4 Mass Spectrometry for Proteomics: Major Application in Food Safety and Quality

The development of molecular medicine is associated with the development of proteomics. In fact, proteomic analyses of early-stage cancers have provided new insights into the changes that occur in the early phases of tumorigenesis and represent a new resource of candidate biomarkers for early-stage disease. Studies that profile proteomic patterns in body fluids also present new opportunities for the development of novel, highly sensitive diagnostic tools for the early detection of cancer (Wulfkuhle et al. 2003).

This is the most relevant and well-known application of proteomics. However, the tremendous improvement in proteomics sensitivity and specificity due to the progress of mass spectrometric methodologies has reached the scientifically and economically important food management chain. The search for markers of authenticity, quality, and safety of food, as well as the discovery of signature peptides in allergonomics and fraud detection has flourished in the last 10 years (Aiello et al. 2011).

5.4.1 Detection of Food Allergens

Food allergies are an increasingly emergent issue in food science and technology due to the marked increase in these allergies recorded among the population. Allergenic food proteins are numerous and heterogeneous, with a genetic polymorphism resulting in several variants for each protein (Picariello et al. 2011; Monaci and Visconti 2009). Up to now, the bottom-up and middle-down (using limited digestion to produce larger peptides) strategies are the most widely used methods for the detection of allergens in food.

Mass spectrometry has allowed the identification of either genes for allergic diseases or allergenic proteins. In this way, MALDI-TOF-MS has been used to quantify gluten gliadins in both processed and unprocessed foods (Camafeita et al. 1997a, b); the procedure is rapid and sensitive with good correlation with data from an immunological assay method. MALDI-TOF-MS can be used as a rapid screening technique for (a) the presence of gliadins in foods by monitoring the occurrence of the protonated gliadin mass pattern in the mass range from 25,000 to 40,000 and (b) the presence of other toxic gluten cereal prolamins fractions, such as barley hordeins, rye secalins, and oat avenins (Camafeita and Mendez 1998) and (Camafeita et al. 1998).

Although MALDI-TOF-MS (Ferranti et al. 2007) and LC-QTOF-MS (Monaci et al. 2010a, b) are widely used instruments for this purpose, other mass analyzers have been applied. For example, Heick et al. (2011) have developed a method based on liquid chromatography–triple-quadrupole mass spectrometry in selected reaction monitoring (SRM) mode for the simultaneous detection of seven allergens in milk, egg, soy, hazelnut, peanut, walnut, and almond. The method was based on extraction of the allergens from the food matrix, followed by an enzymatic digestion with trypsin. The MS method was used to detect all seven allergens from an incurred bread matrix.

In light of the bibliography, proteomics MS-based methods for allergenic protein identification, characterization, and quantification deserve a great deal of attention. New MS analyzers such as orbitrap instruments are being focused on analysis of intact protein or the use of accurate peptide mass for high-throughput analysis (Monaci et al. 2011).

5.4.2 Detection of Pathogens

The first attempts to identify microorganisms using mass spectrometry were performed as early as 1975 (Anhalt and Fenselau 1975). However, these experiments suffered from irreproducible results due to the variabilities caused by growth conditions and media. Only with the discovery of MALDI-TOF MS in the 1980s did the analysis of relatively large biomolecules, including larger ribosomal proteins, become possible (Hillenkamp and Karas 1990). The latter are less influenced by culture conditions allowing MALDI-TOF MS to be consistently used to differentiate

bacterial species. In recent years, MALDI-TOF MS has been implemented in routine laboratories and utilized as a completely new approach for the identification of bacteria and yeast (Wieser et al. 2012).

Regarding other mass analyzers, Sospedra et al. (2012) have developed a simple, rapid, economic, and effective procedure for the simultaneous analysis of two staphylococcal enterotoxins using a triple-quadrupole instrument. The authors concluded that this detector has many advantages over traditional techniques such as avoiding the necessity of preliminary steps to isolate the bacteria from food, possibility of quantification, and lower limits of detection; moreover, under the proposed conditions, SEA and SEB (enterotoxins A and B) could be directly detected from milk and other food samples.

Melanson et al. (2006) have reported the first application of the MALDI-QqQ to validate an important virulence biomarker of *Candida albicans* employing an amine-specific isotopic labeling approach; the technique was validated using standard peptides differentially labeled at known concentrations.

5.4.3 Food Processing

Proteins are largely responsible for the characteristics of many food products during the manufacturing process: physicochemical properties, such as viscosity, thermal conductivity, and vapor pressure, but also nutritional and sensory properties depend on their protein composition and content. This manufacturing process induces significant changes including chemical modification, heat-shock protein responses, and proteolytic degradation (Gašo-Sokač et al. 2010).

As an example, many different thermal treatments are used in milk products, typically for the improvement of shelf life, which can, however, potentially affect the nutritional and functional qualities of milk proteins. Structurally sensitive techniques must rely on the detection of intact proteins by MS; for example, MALDI-ToF-MS has been applied to unfractionated milk for the characterization of milk-processing history with some success (Holland et al. 2011). In this work 2DE coupled with MALDI-TOF MS was used to examine the effects of storage at elevated temperature on UHT milk.

Zhang et al. (2012) developed a reliable LC-triple quadrupole method based on tryptic product peptides for determination of total α-lactalbumin in infant formulas and whey protein concentrates. The quantitation of the α-lactalbumin content was carried out by digesting the infant formula or whey protein concentrates followed by MRM-based LC–MS/MS analysis at the peptide level. An additional advantage of the current method was a measure of the whole bovine α-lactalbumin including the native form and thermally induced denatured form. Finally, it was successfully applied to determine bovine α-lactalbumin in 21 different infant formulas and eight whey protein concentrates. Because the established method could determine native and thermal denatured bovine α-lactalbumin, as well as soluble and therefore functional bovine α-lactalbumin, its application promoted the development of nutrient

investigation and quality control of infant formulas and other dairy products containing α-lactalbumin.

Protein composition of other foods such as meat and meat products are more complex, and the change of physicochemical properties during processing depends on more than one highly abundant protein (Lametsch et al. 2002). When looking for meat quality indicators, researchers often wonder which compounds can best be used to represent them. Inasmuch as proteins make up a considerable part of the meat basic composition and changes are associated with its quality, it is not surprising that these compounds are frequently looked to as a source of possible indicators/markers of meat quality (Bendixen 2005).

Bernevic et al. (2011) reported the identification and structural characterization of post-mortem degradation and oxidation of myofibrillar proteins using high-resolution mass spectrometric proteomics. The combination of 2D-PAGE and FTICR-MS was found to be a powerful approach for identification of muscle protein degradation products, providing identification of several truncation forms of creatine kinase and troponin T.

5.4.4 Food Adulteration

Proteomics approaches have also been applied to assess other important points included in food quality, because protein profiling can give useful information on food origin or adulteration. Protein profiling with MS can search for biomarkers that permit the characterization of food samples according to their origin (Herrero et al. 2012).

In this sense, Di Girolamo et al. (2012) analyzed honeys of different origin (from chestnut, acacia, sunflower, eucalyptus, and orange) for their proteome content, in order to see if any plant proteins present would allow the proteo-typing of these different varieties. All bands visible in the SDS-PAGE profile of each type of honey were eluted, digested, and identified by mass spectrometry in a hybrid LTQ-orbitrap instrument. It turned out that all proteins identified were not of plant origin but belonged to the *Apis mellifera* proteome. Among the total proteins identified (eight, but only seven as basic constituents of all types of honey) five belonged to the family of major royal jelly proteins, and were also the most abundant ones in any type of honey. It thus appears that honey has a proteome resembling the royal jelly proteome (but with considerably fewer species), except that its protein concentration is lower by three to four orders of magnitude as compared to royal jelly. Attempts at identifying additional plant (pollen, nectar) proteins via peptidome analysis were unsuccessful.

The study of proteins can be also useful to detect adulterations in food products; the aim of these applications usually is the detection of proteins that are not a part of the proteome of the studied product. An example of this approach was the study of Angeletti et al. that examined the capabilities of MALDI-TOF-MS for the characterization of water-buffalo milk and mozzarella cheese to detect possible fraudulence in mozzarella cheese production (Angeletti et al. 1998).

Another example was the development of an untargeted LC-QTOF-MS method for protein analysis in skimmed milk powder (Cordawener et al. 2009). The significantly lower price of other vegetable-protein preparations induces the partial adulteration of the dairy product with soy or pea proteins. It was demonstrated that this procedure detected the presence of adulterations in the milk powder based on differential peptide profiling.

5.5 Mass Spectrometry-Based Proteomics Future Perspectives

Nutrition is still an expanding field for proteomics compared to well-established clinical and medical applications. The success of proteomics in nutrition will depend on multiple factors; one of them concerns the analytical strategies.

In proteomics, MS as a standalone technique or combined with LC has become widespread. However, there is an evident need to develop improved technologies to become a reality for the routine analysis for proteome research, including improvements in the resolution of peptides to provide increased protein coverage. In any case, MS will continue being essential for the systematic investigation in proteomics; in this sense, conventional mass spectrometers as quadrupoles or ion traps are replaced by the more sophisticated and compact mass spectrometers, most of them hybrid instruments in a combination of two or more analyzers.

New applications of proteomics technologies are expected in food safety and quality; as an example, although MS-based proteomics have proven to be a very useful tool for the identification, characterization, and detection of food allergens, there are still some issues that have not been successfully resolved, such as the development of MS-based methods for the simultaneous determination of multiple food allergens in food products and commodities.

References

Aebersold R, Mann M (2003) Mass spectrometry-based proteomics. Nature 422:198–207

Aerbersold R, Goodlett DR (2001) Mass spectrometry in proteomics. Chem Rev 101:269–295

Aiello D, De Luca D, Gionfriddo E, Naccarato A, Napoli A, Romano E, Russo A, Sindona G, Tagarelli A (2011) Multistage mass spectrometry in quality, safety and origin of foods. Eur J Mass Spectrom 17:1–31

Angeletti R, Gioacchini AM, Seraglia R, Piro R, Traldi P (1998) The potential of matrix-assisted laser desorption/ionization mass spectrometry in the quality control of water buffalo mozzarella cheese. J Mass Spectrom 33:525–531

Anhalt JP, Fenselau C (1975) Identification of bacteria using mass spectrometry. Anal Chem 47:219–225

Bendixen E (2005) The use of proteomics in meat science. Meat Sci 71:138149

Bernevic B, Petre BA, Galetskiy D, Werner C, Wicke M, Schellander K, Przybylski M (2011) Degradation and oxidation postmortem of myofibrillar proteins in porcine skeleton muscle revealed by high resolution mass spectrometric proteome analysis. Int J Mass Spectrom 305:217–227

Cairns DA (2011) Statistical issues in quality control of proteomic analyses, good experimental design and planning. Proteomics 11:1037–1048
Camafeita E, Mendez E (1998) Screening of gluten avenins in foods by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Mass Spectrom 33:1023–1028
Camafeita E, Alfonso P, Acevedo B, Mendez E (1997a) Sample preparation optimization for the analysis of gliadins in food by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Mass Spectrom 32:444–449
Camafeita E, Alfonso P, Mothes T, Mendez E (1997b) Matrix-assisted laser desorption/ionization time-of-flight mass spectrometric micro-analysis: the first non-immunological alternative attempt to quantify gluten gliadins in food samples. J Mass Spectrom 32:940–947
Camafeita E, Solis J, Alfonso P, Lopez JA, Sorell L, Mendez E (1998) Selective identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of different types of gluten in foods made with cereal mixtures. J Chromatogr A 823:299–306
Careri M, Bianchi F, Corradini C (2002) Recent advances in the application of mass spectrometry in food-related analysis. J Chromatogr A 970:3–64
Chen CH (2008) Review of a current role of mass spectrometry for proteome research. Anal Chim Acta 624:16–36
Cordawener JHG, Luykx DMAM, Frankhuizen R, Bremer MGEG, Hooijerink H, America AHP (2009) Untargeted LC-Q-TOF mass spectrometry method for the detection of adulterations in skimmed-milk powder. J Sep Sci 32:1216–1223
Cornish TJ, Cotter RJ (1993a) Tandem time-of-flight mass spectrometer. Anal Chem 65:1043–1047
Cornish TJ, Cotter RJ (1993b) Collision-induced dissociation in a tandem time-of-flight mass spectrometer with two single-stage reflectrons. Org Mass Spectrom 28:1129–1134
Cottrell JS (1994) Protein identification by peptide mass fingerprinting. Pept Res 7:115.124
D'Alessandro A, Zolla L (2012) We are what we eat: food safety and proteomics. J Proteome Res 11:26–36
Di Girolamo F, D'Amato A, Righetti PG (2012) Assessment of the floral origin of honey via proteomic tools. J Proteomics 75:3688–3693
Domon B, Aaebersold R (2006) Mass spectrometry and protein analysis. Science 312:212–217
Emmet MR, Caprioli RM (1994) Microelectrospray mass spectrometry: ultra high-sensitivity analysis of peptides and proteins. J Am Soc Mass Spectrom 5:605–613
Eng JK, McCormack AL, Yates JR (1994) An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. J Am Soc Mass Spectrom 5:976–989
Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM (1989) Electrospray ionization for mass spectrometry of large biomolecules. Science 246:64–71
Ferranti P, Mamone G, Picariello G, Addeo F (2007) Mass spectrometry analysis of gliadins in celiac disease. J Mass Spectrom 42:1531–1548
Fountoulakis M, Juranville JF, Roeder D, Evers S, Berndt P, Langen H (1998) Reference map of the low molecular mass proteins of Haemophilus influenzae. Electrophoresis 19:1819–1827
Garbis S, Lubec G, Fountoulakis M (2005) Limitations of current proteomics technologies. J Chromatogr A 1077:1–18
Gašo-Sokač D, Kovač S, Josić D (2010) Application of proteomics in food technology and food biotechnology: process development, quality control and product safety. Food Technol Biotechnol 48:284–295
Gingras AC, Aebersold R, Raught B (2005) Advances in protein complex analysis using mass spectrometry. J Physiol 563:11–21
Glish GL, Burinsky DJ (2008) Hybrid mass spectrometers for tandem mass spectrometry. J Am Soc Mass Spectrom 19:161–172
Griffin TJ, Xie H, Bandhakavi S, Popko J, Mohan A, Carlis JV, Higgins L (2007) iTRAQ reagent-based quantitative proteomic analysis on a linear ion trap mass spectrometer. J Proteome Res 6:4200–4209
Guerrera IC, Kleiner O (2005) Application of mass spectrometry in proteomics. Biosci Rep 25:71–93

Hager JW (2004) QTRAP™ mass spectrometer technology for proteomics applications. Drug Discov Today Targets 3:31–36
Han X, Aslanian A, Yates JR (2008) Mass spectrometry for proteomics. Curr Opin Chem Biol 12:483–490
Heick J, Fischer M, Pöpping B (2011) First screening method for the simultaneous detection of seven allergens by liquid chromatography mass spectrometry. J Chromatogr A 1218:938–943
Herrero M, Simó C, Garcia-Cañas V, Ibáñez E, Cifuentes A (2012) Foodomics: MS-based strategies in modern food science and nutrition. Mass Spectrom Rev 31:49–69
Hillenkamp F, Karas M (1990) Mass spectrometry of peptides and proteins by matrix-assisted ultraviolet laser desorption/ionization. Methods Enzymol 193:280–295
Holland JW, Gupta R, Deeth HC, Alewood PF (2011) Proteomic analysis of temperature-dependent changes in stored UHT milk. J Agr Food Chem 59:1837–1846
Hunt DF, Yates JR, Shabanowitz J, Winston S, Hauer CR (1986) Protein sequencing by tandem mass spectrometry. Proc Natn Acad Sci U S A 83:6233–6237
Ji QC, Rodila R, Gage EM, El-Shourbagy TA (2003) A strategy of plasma protein quantitation by selective reaction monitoring of an intact protein. Anal Chem 75:7008–7014
Karas M, Hillenkamp F (1988) Laser desorption ionization of proteins with molecular mass exceeding 10,000 Daltons. Anal Chem 60:2299–2301
Klose J (1975) Protein mapping by combined isoelectric focusing and electrophoresis of mouse tissues. A novel approach to testing for induced point mutations in mammals. Humangenetik 26:231–243
Lametsch R, Roepstorff P, Bendixen E (2002) Identification of protein degradation during post-mortem storage of pig meat. J Agr Food Chem 50:5508–5512
Lane CS (2005) Mass spectrometry-based proteomics in the life sciences. Cell Mol Life Sci 62:848–869
Le Blanc JC, Hager JW, Ilisiu AM, Hunter C, Zhong F, Chu I (2003) Unique scanning capabilities of a new hybrid linear ion trap mass spectrometer (QTRAP) used for high sensitivity proteomics applications. Proteomics 3:859–869
Liebler DC, Yates JR (2002) Introduction to proteomics. Tools for the new biology. Humana Press, Totowa
Macek B, Waanders L, Olsen JV, Mann M (2006) Top-down protein sequencing and MS3 on a hybrid linear quadrupole ion trap-orbitrap mass spectrometer. Mol Cell Proteom 5:949–958
Makarov A (2000) Electrostatic axially harmonic orbital trapping: a high-performance technique of mass analysis. Anal Chem 72:1156–1162
Mann M, Hendrickson RC, Pandey A (2001) Analysis of proteins and proteomes by mass spectrometry. Annu Rev Biochem 70:437–473
Marshall AG, Hendrickson CL (2008) High-resolution mass spectrometers. Annu Rev Anal Chem 1:579–599
Melanson JE, Chisholm KA, Pinto DM (2006) Targeted comparative proteomics by liquid chromatography/matrix-assisted laser desorption/ionization triple-quadrupole mass spectrometry. Rapid Comm Mass Spectrom 20:904–910
Michalski A, Damoc E, Hauschild JP, Lange O, Wieghaus A, Makarov A, Nagaraj N, Cox J, Mann M, Horning S (2011) Mass spectrometry-based proteomics using Q Exactive, a high-performance benchtop quadrupole Orbitrap mass spectrometer. Mol Cell Proteom 10: M111.011015-(1–15)
Monaci L, Visconti A (2009) Mass spectrometry-based proteomics methods for analysis of food allergens. TrACs Trends Anal Chem 28:581–591
Monaci L, Losito I, Palmisano F, Visconti A (2010a) Identification of allergenic milk proteins markers in fined white wines by capillary liquid chromatography-electrospray ionization-tandem mass spectrometry. J Chromatogr A 1217:4300–4305
Monaci L, Nørgaard JV, van Hengel AJ (2010b) Feasibility of a capillary LC/ESI-Q-TOF MS method for the detection of milk allergens in an incurred model food matrix. Anal Methods 2:967–972

Monaci L, Losito I, Palmisano F, Godula M, Visconti A (2011) Towards the quantification of residual milk allergens in caseinate-fined white wines using HPLC coupled with single-stage Orbitrap mass spectrometry. Food Add Contam A 28:1304–1314

Narasimhan C, Tabb DL, VerBerkmoes NC, Thompson MR, Hettich RL, Uberbacher EC (2005) MASPIC: intensity-based tandem mass spectrometry scoring scheme that improves peptide identification at high confidence. Anal Chem 77:7581–7593

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

Picariello G, Mamone G, Addeo F, Ferranti P (2011) The frontiers of mass spectrometry-based techniques in food allergenomics. J Chromatogr A 1218:7386–7398

Ruan Q, Ji QC, Arnold ME, Griffith Humphreys W, Zhu M (2011) Strategy and its Implications of protein bioanalysis utilizing high-resolution mass spectrometric detection of intact protein. Anal Chem 83:8937–8944

Scigelova M, Makarov A (2006) Orbitrap mass analyzer -overview and applications in proteomics. Prac Proteom 1–2:16–21

Sénéchal S, Kussmann M (2011) Nutriproteomics: technologies and applications for identification and quantification of biomarkers and ingredients. P Nutr Soc 70:351–354

Shen TL, Noon KR (2004) Liquid cromatography-mass spectrometry and tandem mass spectrometry of peptides and proteins. In: Aguilar MI (ed) HPLC of peptides and proteins. Methods and protocols. Humana Press, Totowa, pp 111–139

Smith RD, Loo JA, Edmonds CG, Barinaga CJ, Udseth HR (1990) Sensitivity considerations for large molecule detection by capillary electrophoresis-electrospray ionization mass spectrometry. J Chromatogr 516:157–165

Sospedra I, Soler C, Mañes J, Soriano JM (2012) Rapid whole protein quantitation of staphylococcal enterotoxins A and B by liquid chromatography/mass spectrometry. J Chromatogr A 1238:54–59

Westermeier R, Marouga R (2005) Protein detection methods in proteomics research. Bioscience Rep 25:19–32

Wieser A, Schneider L, Jung J, Schubert S (2012) MALDI-TOF MS in microbiological diagnostics—identification of microorganisms and beyond (mini review). Appl Microbiol Biotechnol 93:965–974

World Food Summit (1996) http,//www.fao.org/wfs/index_en.htm

Wulfkuhle JD, Liotta LA, Petricoin EF (2003) Early detection: proteomic applications for the early detection of cancer. Nat Rev Cancer 3:267–275

Yates JR III (2004) Mass spectral analysis in proteomics. Annu Rev Biophys Biomol Struct 33:297–316

Yates JR III, Cociorva D, Liao J, Zabrouskov V (2006) Performance of a linear ion trap-orbitrap hybrid for peptide analysis. Anal Chem 78:493–500

Zhang J, Lai S, Zhang Y, Huang B, Li D, Ren Y (2012) Multiple reaction monitoring-based determination of bovine α-lactalbumin in infant formulas and whey protein concentrates by ultra-high performance liquid chromatography–tandem mass spectrometry using tryptic signature peptides and synthetic peptide standards. Anal Chimica Acta 727:47–53