

The role of direct high-resolution mass spectrometry in foodomics

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Abstract Foodomics has been defined as a global discipline in which advanced analytical techniques and bioinformatics are combined to address different questions in food science and nutrition. There is a growing number of works on the development and application of non-targeted omics methods in foodomics, which reflects that this emerging discipline is already considered by the scientific community to be a valuable approach to assess food safety, quality, and traceability as well as for the study of the links between food and health. As a result, there is a clear need for more rapid, high-throughput MS approaches for developing and applying non-targeted studies. Nowadays, direct MS analysis is one of the main choices to achieve high throughput, generating a set of information from the largest possible number of samples in a fast and straightforward way. The use of high- and ultrahigh-resolution MS greatly improves the analytical performance and offers a good combination of selectivity and sensitivity. By using a range of methods for direct sample introduction/desorption/ionization, high-throughput and non-target analysis of a variety of samples can be obtained in a few seconds by HRMS analysis. In this review, a general overview is presented of the main characteristics of direct HRMS-based approaches and their principal applications in foodomics.

Keywords Foodomics · High-resolution mass spectrometry · Direct MS · Food analysis · Food bioactivity

Introduction

Food analysis is a field in continuous progress, which is particularly driven by consumers' rising demands for food safety and quality. Food safety assessment involves the examination of food for the presence of microbial hazards induced by bacteria, viruses, parasites, toxigenic molds, or microalgae, and also for the presence of chemical compounds such as agrochemicals, environmental contaminants, veterinary drugs, and allergens. Food quality focuses on the evaluation of those attributes and properties that make food acceptable to consumers. In an effort to improve food safety and quality, regulatory authorities have introduced the concept of traceability, which ensures control and management through all stages of food production, processing, and distribution [1]. Effective traceability systems help in the prevention of fraudulent practices which, in most cases, focus on illicit economic gain. Such practices may include adulteration, mislabeling, false use of geographical indications, and deliberate fraudulent practices (production, processing, and distribution), among others, that have direct implications for the quality and the safety of foods. Verification of compliance with food regulations in terms of food safety and quality has been traditionally approached using targeted analysis. This analytical strategy involves a combination of procedures for sample preparation and the subsequent analysis of one or a small number of compounds (pesticides, toxins, proteins, DNA, etc.) from a complex mixture. However, a major requirement to effectively apply the targeted approach is to know the identity of the compound before it can be detected, excluding its application to the analysis of unknown compounds. Considering that

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limitation and the increasing requirements from administrative authorities for control of food safety and quality with scientifically based guarantees, the development of novel and complementary non-targeted analytical methodologies is highly advisable. Non-targeted methods combined with suitable chemometric tools have the potential to improve the breadth of traditional targeted analysis and open up new prospects for novel applications, providing the opportunity to explore formerly unanswered problems relevant to food science [2–4]. A good example of this revolutionary view of food analysis is the emerging discipline of foodomics. This discipline studies the food and nutrition domains through the application and integration of advanced omics technologies to improve consumers' well-being, health, and confidence [5]. Thus, foodomics can be conceptualized as a global discipline in which advanced analytical techniques and bioinformatics are combined to address different issues in food science and nutrition. Another area in which foodomics has found an important application is the investigation of the links between food and health [6–10]. In this regard, the growing number of published reports on the development and application of non-targeted methods in foodomics studies reflects that this emerging area is already considered by the scientific community to be a valuable approach to assess food safety, quality, and to study the links between food and health [11, 12].

Hyphenated mass spectrometry techniques such as gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS), and, to a lesser extent, capillary electrophoresis-mass spectrometry (CE-MS) have achieved great success in non-targeted applications. Chromatographic or electrophoretic separation reduces ion suppression effects originating from the sample matrix. However, the major drawback of hyphenated MS-based techniques lies in their limitation to analyze large sets of samples to detect changes in composition in a fast and simple way. There is a clear need for more rapid, high-throughput MS approaches for non-targeted studies. Nowadays, direct MS analysis is the method of choice to achieve the maximum high-throughput production of information from the largest possible number of samples. In direct MS analysis, any separation step prior to MS detection is avoided and thus direct analysis of samples (previously processed or not) is carried out. Without a chromatographic or electrophoretic separation minimal data pre-processing is required, thereby simplifying the analysis and increasing the speed, which makes it particularly attractive when dealing with large sample sets.

The use of high-resolution mass spectrometry (HRMS) greatly improves the analytical performance and offers a good combination of selectivity and sensitivity. High mass resolution and mass accuracy together with measured relative isotopic abundance ratios are used to tentatively identify elemental formulae of thousands of different compounds. In practice, comparison against a database of accurate masses is also used to

assign an identity. However, information other than just accurately measured mass is needed to support compound identification. Fragmentation of molecular ions produces a mass spectrum from which the fragments can be used to characterize the molecule of interest. However, MS/MS spectra might vary depending on the instrument used, the collision energy used, the collision gas used, etc. Moreover, fragmentation mass spectra are not always accurate in unequivocal identification of isomeric compounds. NMR, which would allow full structure elucidation, requires large amounts of material and thus low levels of unknown compounds are likely to remain difficult to identify. Matching against standards is also another strategy for identifying unknown compounds. However, in many cases, no authentic standards are available for comparison.

Several reports have recently reviewed the principles and techniques of high-resolution mass spectrometers [13–15]. Fourier transform ion cyclotron mass spectrometry (FTICR MS), especially at high fields, enables reliable assignment of molecular formula for completely unknown small molecules. The new generation of time-of-flight (TOF) instruments as well as the Orbitrap technology offers exceptional sensitivity at high resolution and sub-second scan speeds. By using a range of methods for direct sample introduction/desorption/ionization methods, high-throughput and non-target analysis of a variety of samples can be obtained in a few seconds by HRMS analysis. These direct methods provide innovative ways to utilize available ionization methods and are especially aided by high mass resolution and MS/MS capabilities. In this review, a general overview is presented of the main characteristics of direct HRMS-based approaches and their main applications in foodomics.

Direct injection

By using a variety of direct sample-introduction techniques, non-target analysis can be carried out in a short time by direct MS analysis. Direct infusion (DI) and flow injection (FI) are the main techniques used to introduce liquid samples into the mass spectrometer. While DI refers to the continuous ionization of a static sample, FI consists of injecting a sample into a continuous stream of organic phase flowing to the ionization interface. Among atmospheric pressure ionization (API) techniques, electrospray ionization (ESI) ion source and, to a lesser extent, atmospheric pressure chemical ionization (APCI) are the techniques typically employed in direct MS analysis using DI and FI [16]. When using both DI and FI sample typically needs to be treated to dissolve the compounds of interest in the appropriate solvent. On the other hand, for the direct analysis of organic compounds in gas-phase samples, proton-transfer reaction mass spectrometry (PTR-MS) is mainly used.

DI-HRMS and FI-HRMS

In the food quality and safety field, much research has been done on wine owing to its economic importance. Cooper and Marshall presented the first attempts to prove the capabilities of DI ESI-FTICR MS to fingerprint wine for classification purposes [17]. At that time, the authors suggested that negative ionization mode was preferable to positive ionization because of a dominant sucrose peak in the latter mode which led to limited mass spectral dynamic range. In a different work, a “metabologographic signature” by DI ESI-FTICR MS of barrel-aged wines was suggested [18] following observation that the geographic origin and the species of oak wood influenced the quality of wines matured in oak barrels. On that occasion, the authors claimed that both positive- and negative-ion data showed the same discrimination capabilities. The same DI ESI-FTICR MS analytical platform was employed to study champagne [19], specifically the differences between the chemical fingerprints of the champagne bulk and the aerosol formed when a myriad of ascending bubbles collapse and radiate a multitude of tiny droplets. As expected, hundreds of chemical components with organoleptic properties or potential to be aroma precursors were observed to be preferentially partitioned in the champagne aerosols rather than in the champagne bulk. In a different report, DI ESI-FTICR MS was applied for wine classification samples obtained from different valleys, years of harvest, and processing techniques [20]. The obtained results reinforced the fact that the percentages of correct discrimination and classification of wines depend on the variability of the samples analyzed and the type of signals used for discrimination/classification. Moreover, the authors underlined the importance of the mathematical model (built with spectral information) to improve the percentages of classification. Fermentation process monitoring as well as the addition of sugar or unfermented must to wine has also been investigated with DI ESI-TOF MS [21]. Recently, FTICR MS spectra of grape extracts and wines were used to obtain complex chemical fingerprints of thousands of compounds [22]; as an example, Fig. 1 shows several of these complex chemical fingerprints. The importance of terroir and vintage effects on wine and grape chemodiversities was demonstrated by DI ESI-FTICR MS; however, the majority of the discriminant masses could not be annotated, exemplifying the yet unknown chemical complexity of grape and wine compositions [22]. Other beverages, such as coffee, have also attracted interest. As an example, DI ESI-FTICR MS was employed to analyze polar compounds from coffee aqueous extracts and to explore the capabilities of the technique as a fast method to predict the blend composition of the two most common varieties of the world’s coffee production (robusta and arabica) [23].

Preliminary evidence of the utility of DI ESI-TOF MS for edible oil authentication purposes was shown in 2002 by

Goodacre et al. [24]. Using minimal sample preparation, the authors clearly separated olive oil from oils commonly used as adulterants. Thereafter, characterization of edible oils by DI ESI-MS has continued to receive considerable interest because of the large increase in demand for high-quality edible oils. Compositional-based fingerprints of vegetable oils (canola, olive, and soybean) by DI ESI-FTICR MS were used to characterize them and to detect and identify adulterants [25]. While fatty acids were found to be the most abundant compounds in the negative-ion mass spectra, triacylglycerols and diacylglycerols dominated the positive-ion mass spectra. DI ESI-FTICR MS has also been applied to the study of rancidity in macadamia nut oil [26]. As expected, different methods of oil extraction and storage resulted in different levels of peroxides in the oil that are associated with rancidity. Moreover, non-target analysis of macadamia nut oil extracts indicated that, together with well-known free radical oxidation of fatty acids, enzymatic activity also played an important role in rancidity and, consequently, in oil quality. DI ESI-QqQ-TOF MS and FIA ESI-QqQ-TOF MS were also employed to analyze the lipid fraction and obtain a classification model to discriminate between different types of hams in relation to the feeding of the pigs [27].

DI ESI-FTICR MS also showed the potential to study genetically modified maize samples [28]. Based on the FTICR MS spectral data obtained in both positive and negative ESI modes, differences between the metabolic profiles of transgenic and wild maize could be highlighted and elementary compositions of specific GMO biomarkers were studied (Fig. 2). Electrophoretic mobilities and m/z values provided by the CE-TOF MS platform helped confirm the identity of various isomeric compounds that could not be unequivocally identified by FTICR MS, such as isomeric compounds.

Direct APCI-HRMS and PTR-HRMS

Dynamic measurements of the gas-phase volatile content are possible with direct APCI-MS and direct PTR-MS. Both methods are based on the ionization by gas-phase ion–molecule reactions followed by MS. The two methods mainly differ in the pressure in the system: atmospheric pressure for APCI-MS and low pressure for PTR-MS. APCI-MS has proved to be a valuable technique for direct monitoring of volatile organic compounds (VOCs). When using APCI for the direct analysis of complex food samples, one can sometimes differentiate overlapping ions by inducing limited fragmentation of the compound by increasing the voltage before the ions enter the mass filters, to obtain more spectral information.

In flavor release applications, traditional GC-MS is time consuming compared to direct APCI-MS. Ashraf et al. demonstrated good correlation between both techniques [29], and showed a rapid determination of key aroma compounds in

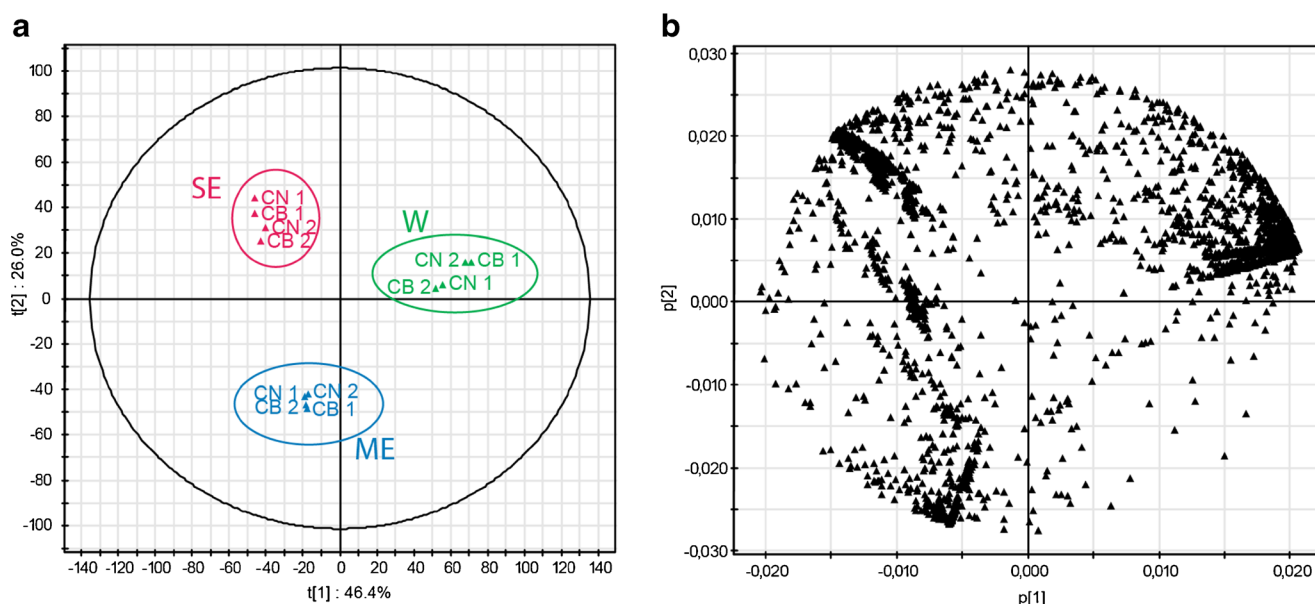


Fig. 1 (a) Scores plot and (b) loading plot of the PCA analysis of the FTICR-MS data for wines (green), skin (pink) and must (blue) extracts of the 2008 vintage from Côte de Nuits (CN) and Côte de Beaune (CB)

locations. The first two components retained 72.4 % of the variation. Reprinted with permission from reference [22]

beer by APCI-MS. This method permitted the time course of flavor compound formation to be followed during fermentation and could also be used to differentiate beers [29]. Other interesting applications can be found in the literature, such as flavor release from French fries [30] and dried red bell peppers [31]. In the latter case, the accurate mass measurements by APCI-TOF MS allowed the differentiation between

compounds with the same nominal mass, but with different elemental compositions.

PTR-MS, originally developed in the mid-1990s [32], is a very interesting technology for fast and sensitive direct measurement of volatile compounds in air, with no pretreatment of the sample. PTR is a form of chemical ionization based on the ionization by gas-phase ion-molecule reactions with the main

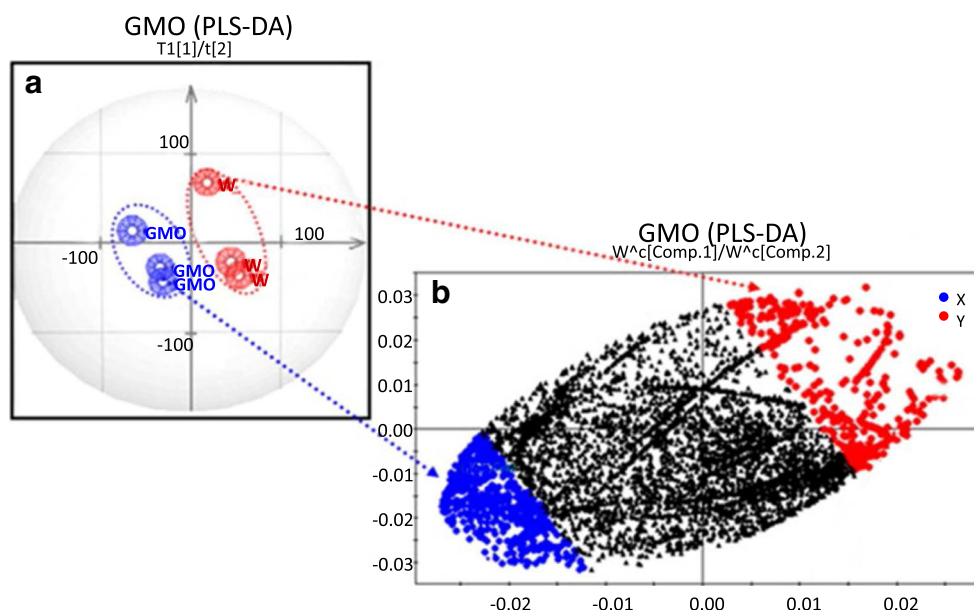


Fig. 2 2PLS-DA model ($Q^2(\text{cum}) = 0.52$ and $R^2(Y) = 0.99$) with six different maize varieties analyzed by DI ESI-FTICR MS. Three GMO and three Wild type (W). The score scatter plot underlines a different pattern for the transgenic (they are represented in blue color) and

isogenic lines (red color). The different properties of the discriminative masses (represented in blue and red in the loading plot) are investigated with MassTRIX (www.masstrix.org). Reprinted with permission from [28]

primary ions H_3O^+ . The ionization principle is governed by the proton affinity (PA) of the sample molecule, which has to be higher than the PA of water, a criterion that is satisfied by most VOCs. This is a soft ionization method, which often does not lead to fragmentation of the product ions. It has been suggested that PTR-MS allows more accurate quantification owing to a controlled and efficient ionization, which also offers higher sensitivity. PTR-MS enables the continuous on-line detection of changes in the composition and quantity of volatiles in food [33], among other types of sample [34]. The application of this technique usually leads to a fingerprint of the emission of VOCs profile from food samples for the study of quality, fermentation, ripening, and processing. As an example, PTR-MS has been used to investigate volatile fingerprints of orange juices subjected to different thermal and pressure treatments [35], classification of milk fats of different qualities [36], prediction of freshness of bread [37], classification of infant formulas according to different brands and physical forms [38], and prediction of sensory profiles of coffee [39]. Special importance has been given to wine [40, 41] owing to the economic importance of this industry.

Quadrupole MS has been employed in most published PTR-MS works. The performance of the first PTR-TOF MS instrument was demonstrated by Blake et al. in 2004 [42]. Since then, improvements in mass resolution, mass range, and LOD have been achieved [43]. Since the pioneering works in 2010 involving the analysis of VOCs by PTR-TOF MS to study the effect of storage conditions of milk on the final quality of cheese [44] and for monitoring of lactic acid fermentation of milk [45, 46], a variety of applications have demonstrated the great potential of this approach in the field of food science and technology. Analysis of VOCs by PTR-TOF MS permitted the discrimination among different olive varieties and stage of ripeness [47], as well as the monitoring of VOC release during post-harvest ripening of different apple cultivars [48]. Classification of dry cured hams produced following the indications of different protected designations of origin (PDOs) [49] and different diet of the pigs [50] was also achieved with PTR-TOF MS. Profiling hundreds of peaks from volatile compounds to study the evolution of volatile compounds during storage of dried porcini was also possible by PTR-TOF MS [51]. Owing to the importance of coffee volatiles in flavor attributes of coffee the suitability of PTR-TOF MS has been demonstrated in several works. Thus, volatile profiles from ground coffees from different origins were obtained by PTR-TOF MS and used to classify samples according to geographic origin [52], to study the evolution of the roasting process [53], and to monitor changes in espresso coffee for investigating the impact of different parameters that affect extraction dynamics of flavor compounds (see Fig. 3) [54]. In another type of practical application, the effect of sugar concentration on the release of volatile compounds both from cereal bars and from mouth during consumption was investigated [55]. The influence of compositional and

structural-mechanical properties of yogurt matrices on the release of flavor volatile compounds has also been studied by PTR-TOF MS [56].

PTR-TOF MS offers interesting advantages, which can be optimally used for online breath analysis [57]. Although there are some established and emerging clinical applications of breath analysis [58], the full potential of PTR-TOF MS to study complex effects of dietary factors on the generation of breath compounds has not yet been exploited. Some works have demonstrated the usefulness of PTR-TOF MS to investigate relationships between pathologies induced by dietary regime and breath composition [59]. Several marker compounds directly related to liver metabolism could be identified in the exhaled breath of rats. Recently, breath analysis in humans by PTR-TOF MS showed that coeliac disease patients on a gluten-free diet presented the same breath profile as healthy ones, demonstrating that at the time of breath analysis they did not show any symptom of the disease [60].

In a recent study, APCI-MS and PTR-MS provided equivalent results for the study of *in vitro* and *in vivo* flavor release [61]. Although a higher degree of fragmentation was observed with PTR-MS, similar response linearity and sensitivities were obtained. One of the drawbacks of PTR-MS is that only those compounds whose PA is larger than that of H_2O can be detected. Recently, with the introduction of the “switchable reagent ionization”, it is possible to switch between H_3O^+ ions ionizing via proton-transfer reactions and NO^+ and O_2^+ , or Kr^+ ions, thereby providing even greater analytical value and extending the range of measurable compounds [62, 63].

Matrix-assisted and matrix-free laser desorption/ionization

MALDI-MS is also a well-established tool and complementary to other soft ionization approaches, such as ESI. Unlike ESI, MALDI affords higher tolerance towards contaminants arising from the sample. Although the major research focus has been on the analysis of large molecules (proteins), MALDI-MS is also applicable to analytes with intermediate and smaller masses. The main limitation in the application of MALDI-MS to analyze small molecules is signal suppression due to matrix background ions. Strategies, such as use of ionic liquid-based matrices [64] or other matrix-assisted compounds, such as 9-aminoacridine [65, 66], have been presented for applications of high-throughput metabolomics. Other approaches involve the use of matrix-free strategies to counteract these interferences. The use of silicon surfaces has also been proved to be effective and simple for high-throughput analysis of small molecular weight compounds [67, 68].

MALDI-MS has been increasingly used in foodomics approaches because of its high sample throughput. Among other applications, it has been used to determine the origin of food

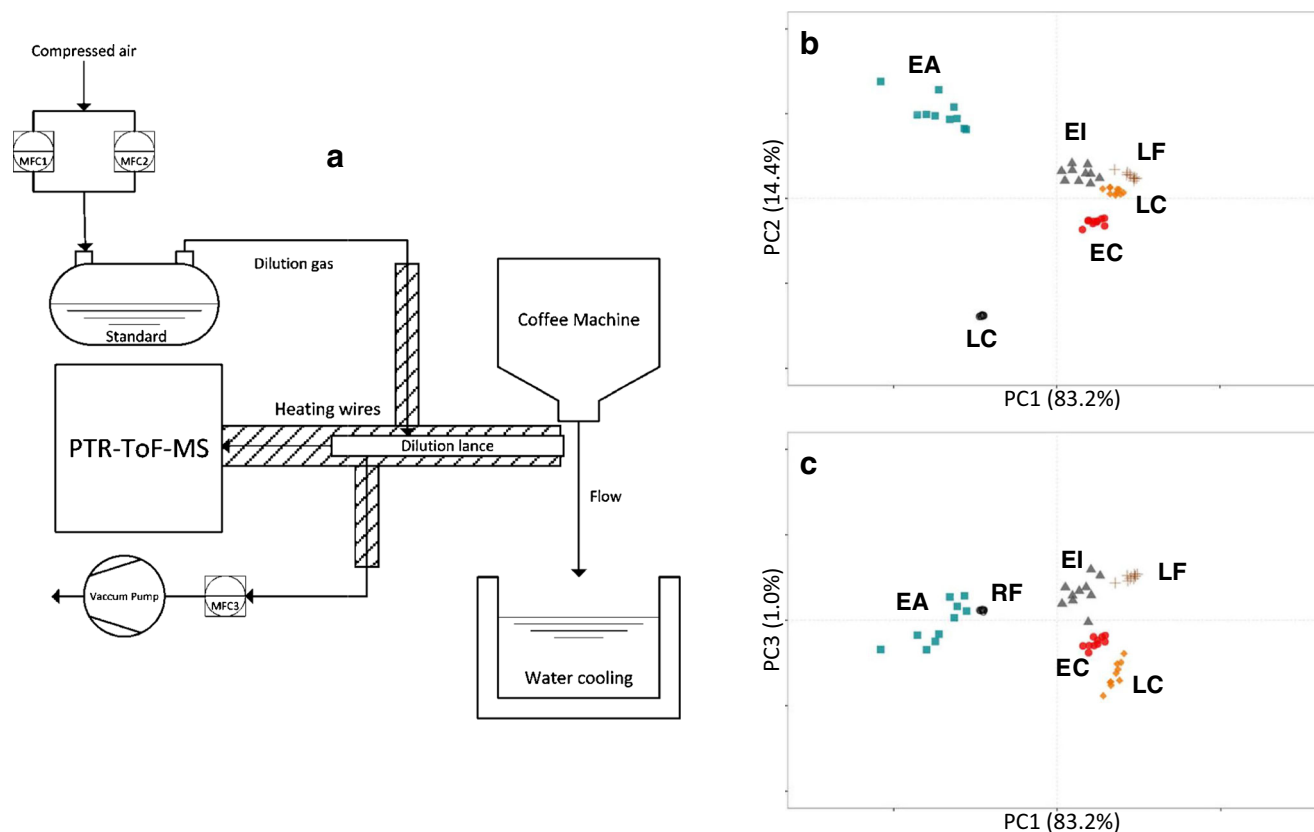


Fig. 3 (a) Set up for sampling VOCs from the coffee flow. Volatiles were introduced into the dilution lancet by a flow created with a vacuum pump and were then diluted 7.5 fold using dried compressed air containing a standard for mass calibration. (b, c) Score plots for the three first dimensions of PCA of the six capsule varieties: Ristretto Forte (RF),

Espresso Intenso (EI), Espresso Alba (EA), Espresso Classico (EC), Lungo Fortissimo (LF), and Lungo Crema (LC)) using the full extraction time corrected by weight of extracted coffee (G, H, I). Reprinted with permission from [54]

[69–71] and food adulterations [72–77]. The discrimination of samples on the basis of their protein fingerprints using MALDI-MS is also an intense area of research [78–80]. For instance, protein and maltooligosaccharide fingerprints from 17 different brands of beer purchased at different time points were obtained with a rapid and simple MALDI-MS method [80]. Beers of the same brand but from different breweries were clearly discriminated and most beers could be assigned to their brands. Milk adulteration and contamination is also a topic of major interest and has been extensively evaluated using MALDI-MS. Thus, MALDI-TOF mass spectra were used to determine and quantify the protein components of various types of milks (from cow, sheep, and goat) with minimal sample pretreatment [73]. By using the whole spectrum of peaks obtained from the analysis, MALDI-TOF MS showed good potential for rapid detection of milk adulteration. Similarly the detection of fraudulent addition of cow milk to sheep and goat milks (of higher value) was investigated by MALDI-TOF MS [75]. Lipid profiles for the analyzed milks showed a different abundance ratio of the ions m/z 703 and 706 which was directly correlated with addition of cow milk into sheep or goat milks. Donkey milk was also analyzed by MALDI-TOF MS in order to detect

contaminants and adulterations with bovine or goat milks [76]. Protein profiles were obtained for goat, cow, and donkey milks and their mixtures in the positive-ion mode without any pretreatment and thus the enhancing speed of the method; unintended contaminations up to a limit of 0.5–2 % were determined and fraudulent mixtures with goat or cow milks were detected using this method [76]. Similar limits of adulteration (ca. 0.5 %) were also obtained through MALDI-TOF MS making it possible to detect adulteration of both donkey and goat milks with other species' milks (cow, ewe, and buffalo) on the basis of their different proteomic profiles [77].

A MALDI-TOF MS method was also developed for the authentication and quality control of 29 hazelnut (*Corylus avellana* L.) cultivars based on the analysis of peptide and protein components [71]. Hazelnut kernel samples were analyzed in the positive-ion mode and acquired in the m/z 3500–15000 range. The unambiguous discrimination of the selected cultivars was achieved on the basis of specific biomarker patterns found in the kernels. Likewise, peptide profiles from the aqueous extract of peanut kernel were analyzed by MALDI-TOF MS [81]. The potential use of peanut extract as a food supplement was revealed owing to its rich peptide content and

low phytate levels [81]. More recently, the effect of encapsulation of fish oils on lipid oxidation was determined by MALDI-TOF MS [82]. Powders obtained from emulsions with different compositions were analyzed for water content, encapsulation efficiency, matrix crystallinity, and oxidation products. A trehalose/sodium caseinate matrix was shown to be the most efficient for microencapsulation of polyunsaturated oils providing a spectrum very similar to the original non-treated oil [82].

Ambient desorption/ionization

Recently, new ambient ionization techniques were introduced to overcome some of the intrinsic limitations of ESI and MALDI, thereby increasing the number of applications that can be addressed using direct MS. The main characteristic of ambient ionization techniques is that they allow direct sampling/ionization of samples in open air or ambient environment with little or no sample preparation. The sample is positioned at the entrance of a mass analyzer in the open environment and molecules released from the sample are ionized and transferred to the mass spectrometer. A variety of methods, based on different desorption/ionization mechanisms, allow the direct MS analysis of a gamut of compounds with a wide range of molecular weights and polarities [83–85]. Among them, direct and desorption electrospray ionization (DESI) and direct analysis in real time (DART) are still the two most prevalent techniques.

DESI-HRMS

In DESI, which is mechanistically related with ESI, electrosprayed aqueous droplets are directed to a surface of interest in air and act as projectiles desorbing ions from the surface as a result of electrostatic and pneumatic forces. Electrical charge applied to the solution produces charged droplets, which in aqueous solutions lead to an excess of hydronium ions (H_3O^+) or hydroxide ions (OH^-), and hence to protonated or deprotonated analytes, which are observable in the positive-ion and negative-ion modes, respectively [86]. This mechanism implies that the solubility of the analytes in the spray solvent is an important consideration in DESI analysis. DESI has shown better performance (i.e., higher tolerance to salts) than direct ESI [87]. It can be applied across an extensive mass range from small molecules to medium size proteins [88] and causes minimum fragmentation. DESI-HRMS has also been demonstrated to be a useful tool for multiresidue analysis of a wide range of agrochemical (insecticides, herbicides, and fungicides) in fruit surfaces [89].

Rapid DESI-TOF MS analysis (ca. 30 s) of sulfur volatiles in several onion varieties made it possible to distinguish phenotypes (tearless and normal) as demonstrated by Joyce et al.

[90]. By using this approach, they confirmed decreased concentrations of the lachrymatory factor 3 and higher concentrations of sulfenic acid condensates in a tearless onion variety. Anthocyanin profile evaluation, used for detection of illicit wine coloring, was also carried out by DESI-TOF MS [91]. A variety of anthocyanins could be detected in red wine samples from different vintages and cultivars, as well as in berries. However, some isobaric compounds remained undistinguished, demonstrating the need for high mass accuracy measurements to improve the applicability of DESI in anthocyanin analysis [91]. Analysis of less polar compounds is also feasible by DESI-MS. Thus, Gerbig and Takáts [92] showed that it was possible to detect triglycerides as alkali metal or ammonium adducts by DESI-Orbitrap MS. Analysis of the triglyceride content in edible oils and fats was carried out by simply dispersing the oil on a glass slide which makes it an attractive approach in high-throughput quality control applications in the food industry [92]. Only a few studies have been undertaken to show the potential of DESI-MS for the investigation of proteins (both standards and biological samples) [93] and protein digests [94, 95]. Recently, Montowska et al. demonstrated the potential of surface desorption/ionization methods together with HRMS for meat protein/peptide analysis for authentication purposes [96]. After assessment of the methodology with standard proteins (myoglobin, troponin C, actin, bovine serum albumin, tropomyosin), ambient DESI and liquid extraction surface analysis (LESA) demonstrated the capability to discriminate subtle spectral differences between samples of meat from closely related species. The detection of unique peptides from the most abundant proteins specific to each species (bovine, horse, pig, chicken, and turkey) was achieved by both DESI- and LESA-Orbitrap. As can be seen in Fig. 4 unequivocal discrimination between species was achieved.

Paper spray [97], a technique with close connections to ESI and nanoelectrospray, is an alternative to DESI for the analysis of surfaces in foods [98]. The electrospray is induced from the sharp tip of a triangular shaped piece of paper containing a small amount of sample by applying a high voltage. Usually, sample is loaded by dropping or by wiping the surface of interest onto the triangular paper. It has also been modified to directly analyze leaves of plants. Following this approach, major sweet glycosides and their oxidation products were identified from a fresh triangular piece of stevia leaf by using an Orbitrap MS [99]. Among the stevia glycosides identified by MS and MSⁿ, rebaudioside D standard was used for semi-quantitative determinations.

DART-HRMS

In DART, an electrical potential is applied to a heated gas stream (typically helium or nitrogen) to form a plasma of excited-state atoms and ions that desorb low molecular weight

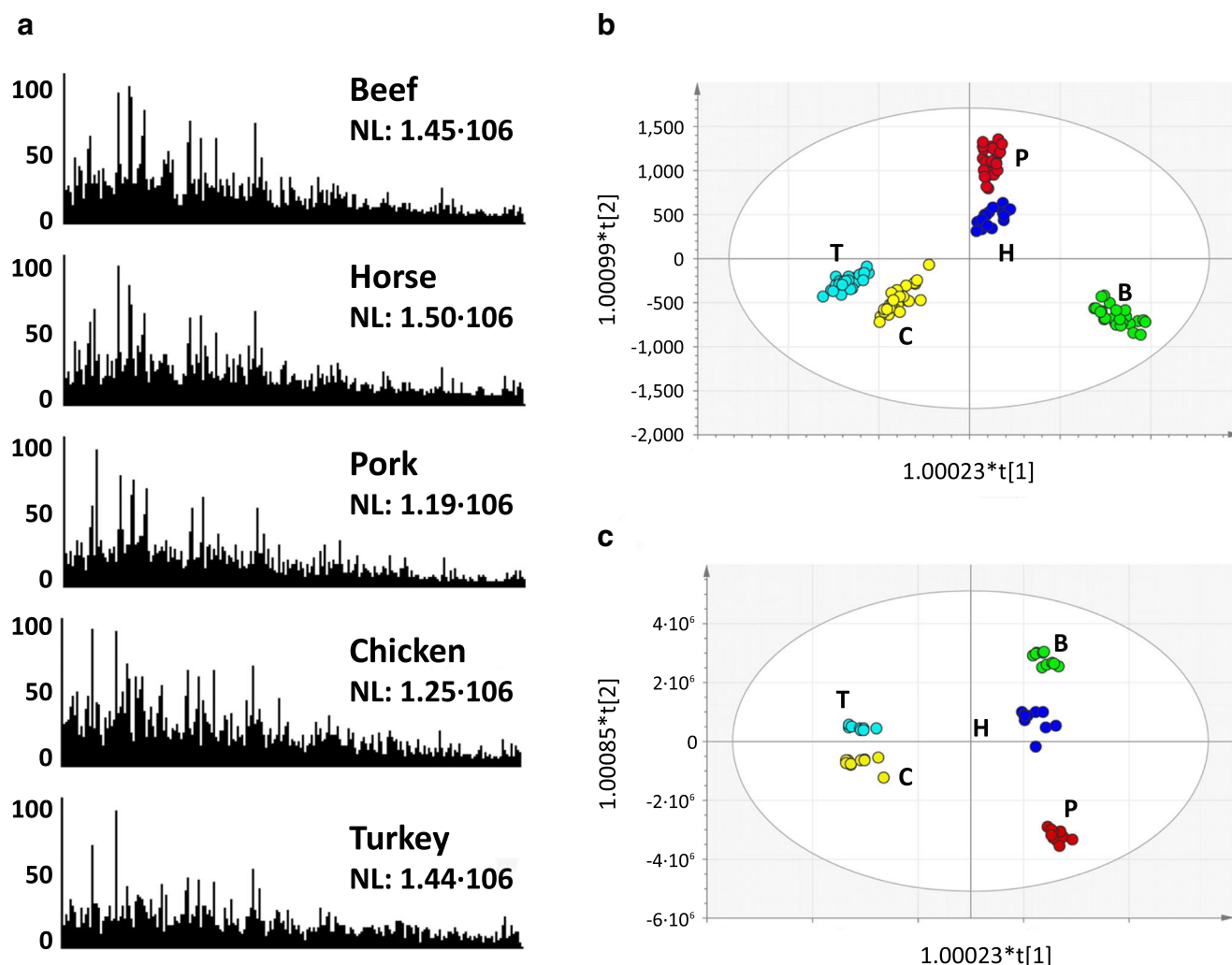


Fig. 4 Differentiation between beef, pork, horse, chicken, and turkey meat. **a** Average mass spectra of meat digest obtained using liquid extraction surface analysis mass spectrometry (LESA-MS); **b** OPLS-DA score plots of data sets collected using DESI-MS in the range of m/z

z 400–1600 ($n=125$); **c** OPLS-DA score plots of data sets collected using LESA-MS in the range of m/z 400–1000 ($n=50$). *B* beef, *H* horse meat, *P* pork, *C* chicken meat, *T* turkey meat. Reprinted with permission from [96]

molecules from the surface of a sample [100]. DART is capable of analyzing low to high polarity compounds (up to 1 kDa) in both negative- and positive-ion modes, either in solid or liquid samples. DART produces relatively simple mass spectra characterized in most cases by $[M+H]^+$ in positive-ion mode and $[M-H]^-$ in negative-ion mode. The majority of systems have interfaced the DART ionization source with TOF MS. DART-MS is gaining importance in foodomics, particularly within the areas of contamination, food packaging, authenticity, and adulteration [101, 102]. DART-HRMS has shown great potential for multiresidue screening. Study of contaminants present in fruits by exposing the peel (without any pretreatment) to DART-HRMS is particularly interesting owing to the high sample throughput and real-time identification of compounds [103]. A total of ten different xenobiotics could be tentatively identified in the different fruit samples. Among them, imazalil was selected to explore quantitative

possibilities of the method and to carry out an unequivocal identification by matching the isotopic cluster with the analytical standard. If no specimen pretreatment is carried out to the surface, it might result in poor sensitivity. Thus, effective contaminant detection was possible by applying swabbing methods on the surfaces of fruits and vegetables [104, 105]. Thus, by spiking different pieces of fruits with a complex mixture of pesticides, it was demonstrated that swabs increased sample throughput by reducing sample preparation and analysis time [104]. A variety of contaminants, such as acrylamide, can also be produced during food processing. Mass spectral fingerprints obtained also by DART-Orbital ion trap MS enabled high-throughput fingerprinting of biscuit samples submitted to different heat-treatments applied during the baking process [106]. Models for prediction of acrylamide were obtained from chemical fingerprints by using partial least square regression (PLSR) analysis to accurately predict

the amounts of acrylamide formed during baking of biscuits under various conditions of time and temperature. DART-Orbitrap MS has also demonstrated potential in the study of other unintended processes, such as lipid oxidation, which may occur during processing/storage of vegetable oils and that can lead to deterioration of both their nutritional and sensory quality [107].

Mislabeled, adulteration, false use of geographical indications, deliberate fraudulent practices, among others, have important economic implications in the food industry; and in this field, DART-HRMS is undergoing rapid development. DART-TOF MS has demonstrated the capability to classify olive oils according to their quality grade, as well as adulteration of extra virgin olive oil with other cheaper oils [108]. In that work, triacylglycerols (TAGs) and TAGs together with polar compounds dominated the spectral profiles of whole oils and methanol-water extracts, respectively [108]. As expected, differentiation of positional isomers of TAGs with two or three different fatty acids was not possible by DART-TOF MS. Analysis of non-polar fractions obtained from cheese by DART-Orbitrap MS enabled fast recognition of cheese adulteration with poor quality vegetable oils. Discrimination was based on detection of compounds arising from oxidation of plant TAGs [109]. Authentication of animal fat through rapid profiling of TAGs was also feasible by DART-TOF MS and multivariate analysis [110]. Unequivocal identification of TAGs detected in the samples and/or the differentiation among their stereoisomers was not possible because of the simultaneous desorption/ionization of all compounds present; nevertheless, the resulting mass spectral fingerprints enabled the creation of predictive models for animal fat classification. Fraudulent practices of animal feeding [111], beer origin recognition [112], and identification of the origins of commercial cubeb fruits [113] were also feasible through chemical fingerprinting by DART-TOF MS and subsequent multivariate analysis. DART-HRMS is also gaining importance in food characterization of complex matrices, such as propolis [114] and mustard [115], as a fingerprint-based approach for fast qualitative evaluation of food products. In the latter work, major glucosinolates found in mustard were tentatively identified as sinigrin and sinalbin. Standards of sinalbin and sinigrin were then used for unambiguous identification and for semiquantitative determination purposes [115].

DAPPI-HRMS

Desorption atmospheric pressure photoionization (DAPPI) [116] is a different ambient ionization method that allows the surface analysis of neutral and non-polar compounds, opening up new possibilities for the analysis of compounds poorly ionized by DESI. DAPPI uses the reactive chemistry of APPI and the analytes from surfaces are desorbed by a heated jet of gas and vaporized spray solvent [117]. DAPPI was shown to have a better matrix tolerance and to cause less

contamination of the ion source when compared with DESI [118]. DAPPI-MS has also been demonstrated to be a suitable technology in environmental and food analysis [119]. However, so far low resolution mass spectrometers have been used together with DAPPI and thus limited information could be obtained from the analysis of food products (fish oil and butter) [120].

Conclusions and final comments

Direct HRMS-based approaches combined with suitable chemometric tools are opening up new prospects in food science. Combination of HRMS with direct sample introduction/desorption/ionization methods is a promising approach in foodomics, especially in food safety, quality, and authenticity evaluation studies. Among direct MS-based approaches, ambient mass spectrometry is a very active area of research in which modifications to existing methodologies are continuously being introduced. Traditional barriers to MS analysis of samples in their native states are being overcome by these strategies, and analysis of surfaces in foods involving minimum sample preparation is now possible by these approaches. Non-destructive and real-time detection of volatile compound by PTR-HRMS for the measurement of flavor in complex food systems or in matrices undergoing time-dependent transformations, or of food mastication, is also a very interesting direct MS-based strategy. However, the inherent complexity of food samples presents significant analytical challenges. With the lack of chromatographic or electrophoretic separation prior to MS analysis, high-resolution mass spectrometers are compulsory for characterization of complex samples.

There are several critical points in direct HRMS-based approaches. Without a preceding separation step, the sample matrix can directly affect desorption and ionization processes. In addition, isobaric interferences in the mass spectrum adversely affect compound identification as well as other aspects of performance. On the other hand, the high throughput and the reduction of sample preparation time are the main advantages of direct HRMS-based approaches over conventional hyphenated techniques. These approaches are advancing the reliability and accuracy of high-throughput non-target direct MS analysis and are just the beginning of a new set of applications in foodomics. In this regard, we foresee the development and application of new direct HRMS methods that can help to improve our understanding of the links between food and health, a topic still to discover by direct HRMS practitioners.

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