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Food quality and authenticity screening *via* easy ambient sonic-spray ionization mass spectrometry

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This review is the first to summarize a decade of studies testing the use of easy ambient sonic-spray ionization mass spectrometry (EASI-MS) and its several sister techniques, Venturi (V-EASI), thermal imprinting (TI-EASI) and Spartan (S-EASI) mass spectrometry in food quality control and authentication. Since minimal or no sample preparation is required, such ambient desorption/ionization techniques have been shown to provide direct, fast and selective fingerprinting characterization at the molecular level based on the pools of the most typical components. They have also been found to be applicable on intact, undisturbed samples or on simple solvent extracts. Fundamentals of EASI-MS and its sister techniques, including mechanisms, devices, parameters and strategies, as well as the many applications reported for food analysis, are summarized and discussed.

1. Introduction

Food is one of the most basic human needs and ensuring food safety and quality is a major health concern.[1,2] This essential source of energy and nutrients may be however subjected to a number of undesirable processes, such as contamination, poisoning, adulteration, fraud and degradation. These processes, intentionally caused or not, may turn food into very undesirable or dangerous products, whose consumption should be avoided.[3] Analytical methodologies are, therefore, essential to monitor food quality and authenticity. These techniques are under constant development in the search for protocols that could provide a food analysis that is fast, simple and as comprehensive as possible, at the most detailed, atomic and molecular levels.[4] Authenticity, chemical composition analysis, shelf life, degradation and the presence of adulterants are among the major reasons to inspect food. These assays may

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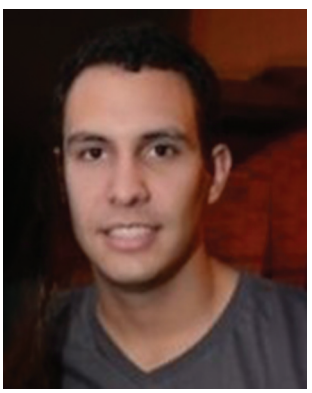

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involve simple techniques, such as those that measure water contents but may also require quite sophisticated, time-consuming and elaborate tests, such as genetic sequencing.[5] Particularly when addressing food authenticity and fraud, profiles of a range of typical constituents or specific biomarkers may be useful to characterize ingredients, processing, geographical origin, production systems, aging and classification. In this scenario, mass spectrometry (MS) has offered a powerful tool, since it possesses a series of beneficial figures of merit, such as great speed of analysis, superior specificity, high sensitivity and the possibility of performing multi-component and profiling analysis at the atomic and molecular levels.

At first, applications of MS on food analysis were restricted by a major MS drawback: the technique was applicable only to molecules that could be brought to the gas phase normally *via* volatilization. MS comprised therefore compounds with relatively low molecular weight (MW) and polarity, medium to high volatility and thermal stability. Many inventions tried to bypass this MS drawback, such as the pioneering work of Morrison and Hercus[6] in which they collected the "breath of apples" in a cold trap and then analyzed it by direct infusion MS. Thus, they could identify a mixture of ten olefins, esters and aldehydes. Components which were larger, thermally unstable and more polar were however excluded from the MS world. Fortunately, in the early 90s, the development of the electrospray ionization technique (ESI)[7] by John Fenn *et al.*, who was awarded the Nobel Prize in 2002, eliminated such a limitation by incorporating nearly all the types of molecules into the MS portfolio. Medium to very polar molecules and those with a high MW, as well as cations and anions, could then be handled by ESI, thus, greatly expanding the applicability of MS in food chemistry.

An additional window of opportunities was opened by the 2nd revolution in MS, which more strongly boosted MS for applications in food chemistry. This revolution was brought by the development of a series of techniques collectively known as ambient desorption/ionization techniques.[8–10] By offering unique approaches for sampling and ionization, ambient MS now provides desirable adjectives for food analysis, such as "simple", "easy to perform", "nearly non-destructive", "without sample preparation", "cost-efficient" and "fast".[11] Although a great variety of ambient MS techniques have already been reported,[8] a selected set of these techniques that includes the two pioneering techniques of desorption electrospray ionization (DESI)[12] and direct analysis in real time (DART),[13] has gained widespread acceptance. For food analysis, easy ambient sonic-spray ionization (EASI)[14] has been tested with excellent results in several applications.

EASI[14–16] is a spray-based technique that employs the action of a polar solvent to promote both desorption and ionization of analytes. It displays a series of characteristics which are beneficial for ambient MS as well as for food analysis. Contrary to other spray based techniques, EASI uses no voltage, electrical discharges or high temperatures for ion production, hence EASI is free from electrical or thermal interferences. EASI droplets seem also to promote a more selective ionization of analyte molecules with reduced solvent and contaminant ionization offering excellent signal to noise (S/N) ratios as well as proper absolute ion abundances.[16] In terms of demands for source mounting, EASI is also attractive since it can be easily mounted using simple parts which are commonly available in MS laboratories. Mounting an EASI source is also simplified by the absence of a voltage power supply. EASI offers therefore a simple, robust and easy to assemble

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and operate ionization device which has been used in direct protocols for food analysis, avoiding time-consuming extraction, derivatization and separation/chromatographic steps usually required by other techniques.[17]

Some reviews have appeared recently on the applications of MS and ambient MS techniques in food analysis. Li *et al.*[18] have reviewed typical applications of ambient MS in food safety and quality control focusing on DESI, DART and extractive electrospray ionization (EESI). Guowang *et al.*[19] have also reviewed the application of ambient MS for direct food analysis, focusing on desorption atmospheric pressure photoionization (DAPPI), DART, DESI, and EESI. Recently, Ibáñez *et al.*[20] have shown the application of ambient MS in food metabolomics focusing on DESI and DART. By reviewing more specifically the applications for food analysis of a single ambient MS technique, Hajslova *et al.*[21] and Guo *et al.*[22] have detailed the use of DART, whereas Nielen *et al.*[23] have concentrated on DESI. We note however that, although EASI has been certainly a central technique in this field, with multiple applications being described, and although it has been applied with emphasis for nearly a decade in food analysis, its use has not been properly reviewed so far.

Celebrating the 10th anniversary of EASI and its sister techniques, this review summarizes therefore its principles and predominant applications in food authenticity and quality control. The major topics that will be covered include food authentication, composition, degradation processes and quality control. In addition, comparisons to other techniques will be sometimes highlighted, thus enabling a better understanding of the advantages and drawbacks of EASI-MS applied to food analysis.

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Dr Rosana M. Alberici received her B.Sc. degree in Chemistry from the Federal University of São Carlos in 1990 and a Ph.D. in Chemistry in 1996 from the University of Campinas – UNICAMP, Brazil. She worked as a postdoctoral fellow at the ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, UNICAMP (1997–2000), where she is currently a Researcher Collaborator (2008–2014). She was a professor of chemistry at private universities (2000–2008). She has also been a visiting research scientist at the Fernandez's Laboratory at the School of Chemistry and Biochemistry, Georgia Institute of Technology, USA (2013–2014). Her research focuses on the area of analytical mass spectrometry, with a special emphasis on ambient ionization applied to lipidomics.

2. Fundamentals of EASI

2.1 Ionization mechanism

The EASI technique was first introduced by Eberlin and co-workers in 2006.[14,16] It is an ambient desorption/ionization technique based on the sonic-spray ionization (SSI) process which was first described by Hirabayashi and co-workers in 1994.[24] The SSI was unique and introduced a new concept of ionization in MS. That is, for the first time in MS history, ions were formed without the assistance of voltage, radiation or heating. In SSI, ions are produced from solutions simply by a process that can be described as "solution shearing" that "explosively" forms very minute droplets. These droplets have a very reduced charge loading ability. Solution shearing leads to a statistically unbalanced distribution of cations and anions and a resulting bipolar stream of charged droplets with a very low charge density is therefore formed with only the assistance of high-velocity compressed nitrogen (or even air). EASI uses therefore this bipolar stream made of SSI droplets to bombard surfaces containing the target analyte molecules (M), which are then transferred to such droplets by a droplet pick-up process. Inside the droplets, the neutral M are ionized either by protonation, deprotonation, cationization or anionization. Because of solvent evaporation and further shrinking, the analyte ions are then ejected from the droplets to the gas phase and subjected to MS analysis. When operated in the positive ion mode, EASI(+) typically forms protonated molecules $[M + H]^+$, sodium $[M + Na]^+$ or potassium $[M + K]^+$ cation adducts. EASI(−) mainly forms deprotonated analyte molecules $[M - H]^-$. Note that, since a voltage-free process is used, $M^{+\bullet}$ or $M^{-\bullet}$ concurrent species, which are sometimes observed in other spray-based voltage-assisted techniques and may disturb M characterization in complex mixtures, should not and have never been observed in EASI-MS experiments.[14,16] Since EASI is based on SSI, which is perhaps the softest ionization technique,[25] it also favors the detection of intact analyte ions. This softness is advantageous for the analysis of fragile molecules or complex mixtures when MS is also used as a separation technique since it provides a direct 1 : 1 molecule-to-ion relationship. Fig. 1 shows the schematic diagrams of EASI,

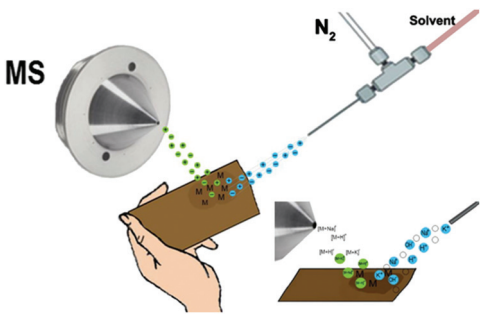

Fig. 1 Schematic diagram for EASI. The insert illustrates the ions which take place in the ionization/desorption process.

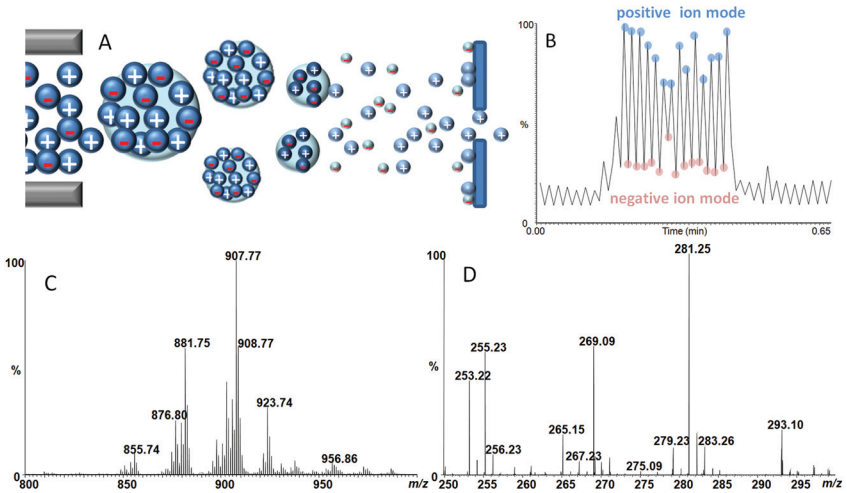

Fig. 2 EASI(±) spectra of olive oil obtained using a Q Exactive (Thermo Fisher Scientific – Germany) mass spectrometer coupled to the Omni Spray 2-D Ion Source (Prosolia – USA). The DESI source was replaced by the EASI source. The charge and ion formations for the SSI process are illustrated (A). The total ion current (TIC) for the analysis of olive oil is shown in (B) using a switch mode detection method in a single EASI experiment, without the need to change the solvent. The positive and negative ion profiles for olive oil are shown in (C) and (D), respectively.

in which the major parts highlight the basic principles of their operation.

The bipolar stream produced by the SSI process reduces or eliminates the need for the use of an acidified or a basified solvent. This feature enables accessing both cations and anions in the same experiment. Fig. 2 illustrates the production of a bipolar stream of charged droplets in SSI, and it shows the possibility of detecting both cations and anions from the same EASI event. That is, a single droplet of olive oil was deposited on a paper surface which was bombarded by the bipolar spray produced by SSI from pure methanol. The mass spectrometer was then rapidly switched from the negative to the positive ion mode. Note the detection of mainly the characteristic pool of triacylglycerols (TAG), which were detected as $[TAG + Na]^+$ and $[TAG + K]^+$ in the positive ion mode. When operated in the negative ion mode, the free fatty acids from the same sample could then be concomitantly detected as $[FFA - H]^-$ anions from the same "EASI cloud".

The major beneficial features of EASI[8] can therefore be summarized as (i) great simplicity, since only methanol and compressed nitrogen or air are required; (ii) ability to produce simultaneously both negatively and positively charged droplets, thus eliminating the need for switching high potentials when changing from EASI(+) to EASI(−), or *vice versa*; (iii) the low charge concentration on the droplets, which seems to reduce solvent and contaminant noise, thus favoring the analyte ions and therefore improving S/N ratios; (iv) the extreme softness of the ionization process which facilitates analysis of fragile molecules and mixtures; (v) no thermal degradation; and (vi) no electrochemical, discharge or oxidation interferences, which are known to occur in voltage-assisted techniques. EASI, due to its low hardware demands and voltage-free features, also seems very suitable for portable mass spectrometers, and seems to be quite comprehensive in regard to matrices since it has been efficiently applied to a variety of different samples, such as perfumes,[26] biodiesel,[27] illicit drugs,[28] fuels,[29] drug tablets,[16] counterfeit bank notes,[30] biological samples,[31] documents[32] and food analysis.

2.2 EASI source optimization

When EASI is employed, it is important to optimize source parameters aiming at the best desorption/ionization performance. The unique characteristics and mechanism of the technique should be always considered. For instance, simply installing a commercial DESI source and then turning the capillary voltage off and increasing the nitrogen pressure for sonic spraying does not guarantee that the best EASI performance will be attained. Fundamental changes in the spray systems are normally required when moving from a DESI to an EASI source.[16,25] Particularly, the internal and external diameters of the spray capillaries and their relative positions should be optimized for SSI and hence for the best EASI performance. This optimization should lead to very minute droplets of the right sizes for best ion splitting and superior analyte ionization which is promoted by EASI by the "solution shearing" process.

As for DESI,[33] the desorption mechanism for EASI is likely to involve a "droplet pick-up" process. The solid/liquid extraction process is driven by surface wetting with simultaneous solvent thin film formation. As a result of spraying, analytes are extracted from the solid phase into the thin liquid film. Subsequent droplet collisions cause the "splashing" of the analytes in the gas phase. The EASI efficiency for analyte desorption depends, therefore, on the efficiency of spray desorption of the analyte from the surface. Geometry parameters, such as (i) the angle of spray incidence, (ii) the angle formed with the analyte surface and the entrance orifice of the mass spectrometer, (iii) the distance between the source and the surface of the spray and (iv) the distance between the surface and the MS

entrance orifice, can therefore significantly affect desorption/ionization efficiencies during EASI-MS analysis. The optimum adjustment is usually found between 30 and 45° for both angles and 1–2 mm for both distances. The composition of the solvent spray, the type of surface and the pressure of the nebulizer gas are also important parameters when developing EASI-MS methods. These variables can affect the stability of the signal and the detectability of the analytes. The characteristics of the surface in which the analyte is placed are crucial for EASI performance, as for most ambient desorption/ionization ambient techniques. The high affinity of the analyte molecules to the surface or too fast sample washing by the spray can significantly reduce sensitivity. Various surfaces have been used, including brown envelope paper, Teflon, glass and thin-layer chromatography plates (TLC) coated with silica gel (when previous TLC separation is employed), but for EASI porous cellulose paper has been found to offer the best surface.[34] The choice of the spray solvent varies in accordance with the polarity and solubility of the analyte, but typically pure methanol or water/methanol (1 : 1) mixtures are used, eventually with the addition of formic acid or ammonium hydroxide. For solvent flow rates, EASI operates in the range of 10 to 30 μL min^{-1}. For the nebulizer gas, consumption of N_2 is typically 2–3 L min^{-1}. Note that for EASI, N_2 can be conveniently replaced by compressed air,[35] since the voltage-free EASI induces no oxidation under an O_2 environment.

2.3 EASI sister techniques

a. V-EASI. Eberlin and coworkers[36] also developed a self-pumping variant of EASI-MS, which eliminated the need for syringe pumping. By taking advantage of the high velocity stream of N_2 used for the sonic-spray, the EASI technique could then be simplified. Such a variant technique uses the Venturi effect, which occurs when a high-velocity fluid flows through a constricted section of a pipe, thus causing a reduction in fluid pressure and the self-pumping effect. Venturi-EASI, that is V-EASI (Fig. 3), therefore uses the effect of a high-velocity sonic stream of nitrogen or air, and it integrates two fundamental steps of ionization: self-pumping of the analyte or solvent solution *via* the Venturi effect and ionization *via* sonic spray. V-EASI is also advantageous since it can be used in dual mode: for spraying/ionizing analyte solutions and for desorbing/ionizing analytes resting on solid surfaces. For liquid samples, the "free" end of the capillary is dipped into the analyte solution, and the Venturi effect is used to pump this solution to the spray region. For analytes resting on surfaces, the Venturi effect is used to pump a solvent forming the typical sonic bipolar spray. This solvent bombards the sample surface, thus causing desorption and ionization of the analyte. The V-EASI has been used for a variety of solutions and solid samples, such as drug tablets, peptides, proteins, crude oil and drugs.[36–39] The V-EASI set up also facilitates real-time, continuous MS monitoring of the changing composition of solutions, for example along chemical reactions.[36] The V-EASI set up as a whole is very suitable for use in portable mass spectrometers, since this source eliminated the need for electrically assisted syringe pumping, demanding no electrical power requirements at all.

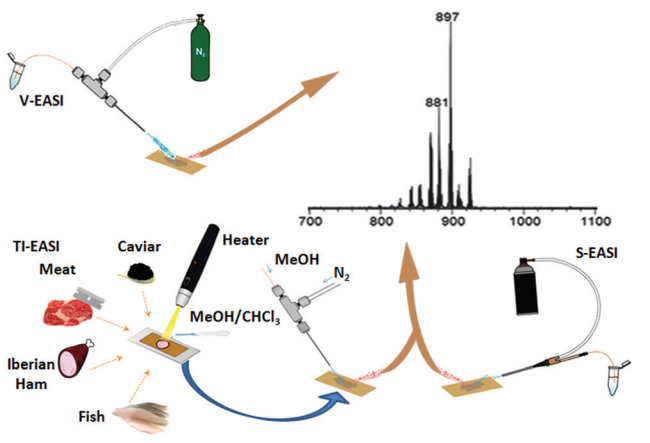

Fig. 3 Schematic representation of EASI sister techniques: V-EASI, TI-EASI and S-EASI.

b. S-EASI. In the search for the simplest and least expensive design of an EASI source, Eberlin and coworkers[35] developed a new variant termed Spartan EASI, that is, S-EASI (Fig. 3). S-EASI operates with no requirements of power supplies, electrical parts or cylinders of compressed gases and pressure regulators, using instead only a can of compressed air to cause both sonic spray and the Venturi self-pumping effect. The spray device was also further simplified since it was built using readily available and inexpensive parts: a surgical 2-way catheter that functions as the T-connector, a fused silica capillary and a simple hypodermic needle. The aerosol dust cleaner can of compressed air therefore replaced the cylinder of compressed N_2 and its gas regulators. Even a fully disposable design for an EASI source can be easily and cheaply assembled using polymeric materials. This should facilitate "in-field" analysis by using portable mass spectrometers, and the source could be discarded and rapidly replaced, thus minimizing carry-over effects.

c. TI-EASI-MS. Eberlin and co-authors[40] have also introduced a variant of EASI based on a very simple lipid extraction procedure prior to analysis. Solvent extraction protocols are usually employed to access the lipid content of a sample.[41] Direct TAG analysis of meats and fats by EASI-MS was performed; however, after the sample surface was washed with a spray solvent, TAG ions escaped detection or were detected at very low abundances, with unacceptable S/N ratios. Thermal imprinting (TI) uses a minimized amount of the solvent to imprint/extract lipids to a piece of paper prior to EASI-MS lipid profiling of meats, fats and fish. A homemade heater made of a 150 W halogen bulb is focused for few seconds (20–90 s) in a small piece of the sample (*ca.* 1 cm^2, 1 mm thick) which is placed over an envelope paper surface. Three or four droplets of the Bligh & Dyer solution[41] (MeOH–$CHCl_3$, 2 : 1 v/v) are dropped into the surface of the sample prior to heating to facilitate the transfer of TAG (Fig. 3). Heating allows the fat content to melt and flow through the sample, getting

imprinted on the paper surface. Direct analysis of the TAG content *via* EASI-MS is then performed on the imprinted material. Different types of matrices have already been evaluated using this technique, as it will be further described. No oxidation products or thermal degradation caused by imprinting were detected when the profiles of TAG were analyzed by TI-EASI(+)-MS. Mailing of the imprinted paper was also proposed for remote TI-EASI-MS analysis of meat and fat samples. The TI was seen to be suitable for the extraction of lipids in few minutes and without the need for the centrifugation or evaporation steps.

3. Applications of EASI-MS in food analysis

3.1 Vegetable oils

a. Oil characterization. Vegetable oils are mostly composed of TAG containing both saturated and unsaturated FA, which can be extracted from various parts of plants, such as seeds, fruits or seedlings. Since they also provide many nutrients, essential fats and vitamins, such oils have been widely used as food ingredients and/or raw materials by food industries. The guarantee of the authenticity of vegetable oils and the proper characterization of their chemical properties are therefore fundamental tasks for food analysis.[42] Traditionally, the analysis of TAG has employed hydrolysis followed by FA derivatization, which generates a mixture of fatty acid methyl esters (FAME). These esterification products are then separated and detected mainly by gas chromatography with flame ionization detection (GC-FID) or mass spectrometry (GC-MS). The sample workup steps are also time-consuming and hard to automate.[43]

Direct EASI(±)-MS analysis without pre-separation or derivatization steps has, however, also been shown to provide comprehensive profiles of TAG and FFA for vegetable oils. The technique provides characteristic profiles of $[TAG + Na]^+$ or $[FFA - H]^-$ ions for each oil type, thus allowing analysis for quality control, origin certification and adulteration detection. Only a single oil droplet is required with no sample preparation. Since EASI uses a quite gentle ionization process, it causes little or no fragmentation of the TAG ions, and therefore diacylglycerols (DAG) as well as monoacylglycerols (MAG), eventually present in the oils, can also be profiled. Characteristic TAG (plus DAG and MAG) fingerprintings have been demonstrated *via* EASI-MS for many different types of oils, such as soybean, palm, olive, hazelnut, grapeseed, andiroba, açaí and castor oils (Fig. 4).[34] For instance, soybean oil shows a unique profile of the $[TAG + Na]^+$ ion dominated by the TAG attributed to PPL (C50:2, *m*/*z* 853), PPO (C50:1, *m*/*z* 855), PLL (C52:4, *m*/*z* 877), PLO (C52:3, *m*/*z* 879), POO (C52:2, *m*/*z* 881), LLLn or OLnLn (C54:7, *m*/*z* 899), LLL or OLLn (C54:6, *m*/*z* 901), OLL or OOLn (C54:5, *m*/*z* 903), OOL (C54:4, *m*/*z* 905) and OOO (C54:3, *m*/*z* 907) (Fig. 4B), where P = palmitic acid, O = oleic acid, L = linoleic acid and Ln = linolenic acid. The profiles of TAG obtained by EASI-MS could closely reflect the relative concentrations of TAG in the original samples[44] and they correspond to the known composition of FA for soybean oil.[45] EASI(±)-MS offers, therefore, a simple and fast method to characterize vegetable oils, as well as other important features and properties, as discussed below.

Besides most classical vegetable oils, EASI(+)-MS fingerprintings have also been used to characterize and control the quality of other "exotic" oils such as those from the Amazon forest.[34,46,47] Oils such as those from andiroba, açaí, urucum and buriti were analyzed and unique profiles of TAG were observed allowing, therefore, for their rapid typification. The EASI(+)-MS profiles for andiroba and açaí oils (Fig. 4C and D) were found to be dominated by TAG containing mainly palmitic, oleic and linoleic acids. The most distinguishing feature of

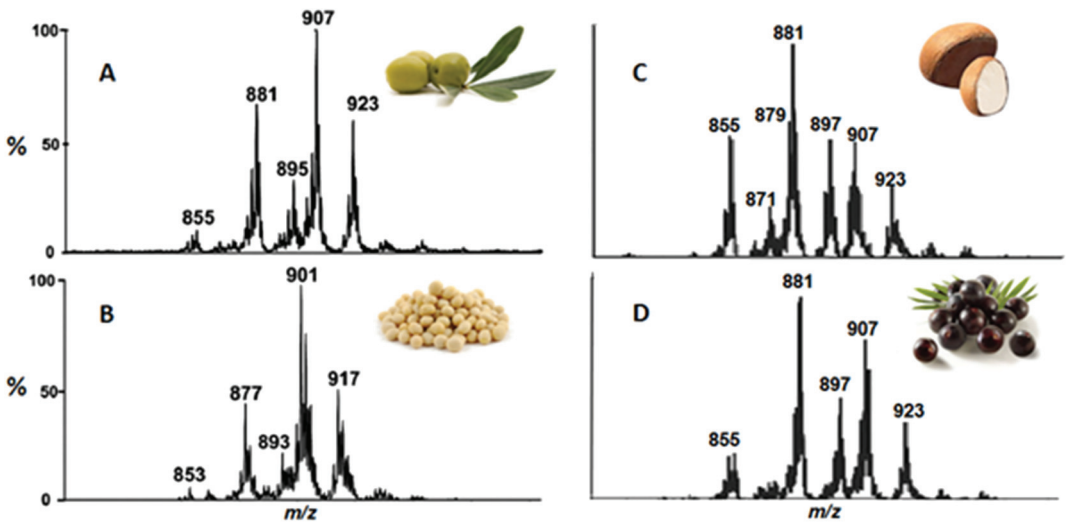

Fig. 4 Representative EASI(+)-MS of vegetable oils. (A) olive oil; (B) soybean oil; (C) andiroba oil; and (D) açaí oil.[51]

the andiroba oil profile was a higher percentage of TAG ions containing palmitic acid, as shown by the abundance of the ion of *m*/*z* 855 (PPO). The oil from açaí is known to be rich in oleic acid, and accordingly the TAG ion of *m*/*z* 907 (OOO) was more abundant in its EASI(+)-MS profile.[34,46] For the buriti oil, the main ion observed was that of *m*/*z* 907 (OOO or SLO) which should function therefore as a biomarker for this unique oil.[48] Coconut oil, which is vastly sold in markets of the Amazon region in Brazil, also displays a very distinct profile in which TAG ions are observed from *m*/*z* 500 up to *m*/*z* 950, with the main $[TAG + Na]^+$ ions being those of *m*/*z* 605 (LaCaCa), 633 (LaLaCa), 661 (LaLaLa or MLCa) and 689 (LaLaM or MMCa), where La = lauric acid, Ca = capric acid and M = myristic acid.[47]

Nine seed oils, from native plants of the Brazilian north and northeast regions, typical of the Brazilian Cerrado have also been characterized by the EASI(±)-MS profiles of TAG and FFA.[48] The species *Jatropha curcas*, *Bombacopsis glabra*, *Capparis flexuosa*, *Siparuna guianensis*, *Moringa oleifera*, *Hibiscus tiliaceus*, *Virola bicuhyba*, *Pouteria caimito* and *Syagrus coronata* were found to exhibit a predominance of TAG composed of the most common FA, that is, palmitic, oleic, stearic and linoleic acids. Two exceptions were clearly observed: *V. bicuhyba* and *S. coronata* seed oils. These unique oils were found to be composed mainly of TAG containing lauric acid. These short-chain TAG make these two oils potential ingredients for spreads and toppings.

Eberlin and co-authors[49] have also examined the maturation of *Jatropha curcas* L. seeds by monitoring changes in the profile of TAG of their oil *via* EASI(+)-MS. The profiles of TAG were observed to substantially change during the maturation and drying processes. The profiles remained however nearly unaltered during storage monitoring. In another study, the *Jatropha curcas* L. oil and its biodiesel were chemically characterized at the molecular level using the profiles of TAG, FFA and FAME obtained by EASI(±)-MS. The overall quality of the *Jatropha curcas* L. biodiesel was found to be quite similar to that of standard biodiesels made from more expensive and more controversial (in terms of their alternative use as food) fresh edible oils.[50]

The profiles of TAG of the soybean oil obtained by EASI(+)-MS have also been compared to those obtained by other techniques, such as MALDI-MS and ESI-MS,[34] as well as GC-FID.[51] The EASI(+)-MS profiles were found to be quite similar to those obtained by MALDI(+)-MS and ESI(+)-MS. Although the three techniques have provided similar information, the use of the ambient MS technique is simpler and requires no sample manipulation. In addition, EASI-MS is easier to perform than the other techniques since it is independent of voltage and temperature and it uses no sample preparation and/or matrix application.

b. Oil authenticity and certification of geographical origin. Adulteration of highly valuable vegetable oils using lower priced oils is a common, widespread and illegal practice. This practice is especially intense for olive oil in Europe,[52,53] where this oil is commonly adulterated with hazelnut oil because of the availability of the latter one and their similar lipid composition.[54] Since the authenticity of one particular type of oil is important for both health and commercial reasons, there is a continued need for improved, rapid, simple and inexpensive methods for oil typification and control of adulteration or counterfeiting. Direct EASI(−)-MS fingerprinting of simple oil extracts allows the prompt differentiation of hazelnut and olive oils, a challenging analytical task because of their close composition of TAG.[55] EASI(−)-MS fingerprinting has also been shown to work as a tool to inspect the geographical origin based on characteristic phenolic constituents, as demonstrated for olive oil.[56] Water : methanol extracts of extra virgin olive oil samples from five different geographical regions (Portugal, Italy, Spain, Greece, and Lebanon) were dried on paper and directly analyzed by EASI(−)-MS. Marker components such as tyrosol and 2-(4-hydroxyphenyl)ethyl acetate were detected as their respective $[M - H]^-$ anions. Using principal component analysis (PCA), Eberlin and co-authors[56] showed that characteristic EASI(−)-MS fingerprinting discriminates olive oil samples from the different geographical regions investigated. The EASI(−)-MS of water : methanol extracts from these oils could also discriminate samples from different genetic varieties with substantial differences regarding the chemotaxonomic marker composition.

Brazil nut oil – an important natural product from the Amazon with several health benefits and applications in food and cosmetic industries[57] – has also been characterized by EASI(+)-MS. Several samples of Brazil nut oil including authentic oils from different geographical origins, commercial oils and oils adulterated with soybean oil were tested.[58] The relative abundance of the ion of *m*/*z* 899, which corresponds to LLLn and/or OLnLn, [C54:7], was used to monitor adulteration through the addition of soybean oil. For this goal, Brazil nut oil was spiked with soybean oil (2, 5, 10, 30 and 50%) and the samples were analyzed by EASI(+)-MS. Indeed, the marker ion of *m*/*z* 899 had it relative abundance steadily increased as a function of blend composition. Consequently, by using this marker ion, an adulteration level as low as 5% of soybean oil in Brazil nut oil could be easily detected by EASI-MS.

c. Edible oil oxidation. Oxidation of edible oils and fats initiates mainly *via* the oxidation of TAG molecules. This process causes a major problem during oil storage, since it may compromise its quality and nutritional value imparting to an oil a typical undesirable rancid aroma.[59] The oxidation of TAG involves a complex set of autocatalytic reactions yielding TAG hydroperoxides as primary products. EASI(+)-MS has successfully been used to monitor such processes, and the results obtained through this methodology were compared to those obtained *via* the classical Rancimat test for oil oxidation.[60] The oxidation levels of oils were continuously determined during the accelerated oxidation process, as well as the final overall oxidation. The detection of TAG mono-, bis- and tris-hydroperoxide products was used for this purpose. The relatively high degree of unsaturation of soybean oil makes this oil quite susceptible to oxidation. Thermally oxidized soybean oil and used frying oil were monitored by EASI(+)-MS, and the characteristic profiles of cations related to hydroperoxide oxidation products as well as DAG ions were observed.[61]

3.2 Emulsifiers

Emulsifiers are one of the few food additives that have multiple functions, acting as an interface between the conflicting components of food, such as water and oil, therefore forming an emulsion. They also improve the texture and shelf-life of many food products. Lecithins, which are used mainly as food emulsifiers and stabilizers, are mixtures of phospholipids (PL), such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Their composition depends on the vegetable source from which they are extracted, and their presence directly influences the characteristics of an emulsion. Because of their complex composition, the analysis of commercial lecithins, as well as the determination of their phospholipid composition, typically involves laborious and expensive work.[62] Eberlin and co-authors[63] showed, however, that EASI(±)-MS can be used as a direct, fast and efficient technique to characterize commercial lecithins and to monitor their quality *via* typical lipid profiles. Samples of commercial lecithins and also standards, refined, deoiled and modified soy lecithin were analyzed, and they showed characteristic profiles of PC and TAG by EASI (+)-MS. EASI(−)-MS complemented the chemical information by providing profiles of PE, glycophospholipids (GPL) and FFA.

Eberlin and co-authors[63] have also compared the EASI (+)-MS and MALDI(+)-MS performances for the analysis of commercial lecithins. The EASI(+)-MS could detect ions related to TAG, PL, lysophospholipids (LPL) and GPL, whereas MALDI (+)-MS detected only PL and GPL ions. A drawback of MALDI (+)-MS is the matrix interference, which complicates the detection of ions lighter than *m*/*z* 600, such as those of LPL. We also observed the absence of TAG and phosphatidylethanolamine (PE) ions in the MALDI spectra, and have attributed this failure to the prone ionization of PC and GPL molecules when using the DHB matrix. EASI(+)-MS therefore provides superior compositional information when compared to MALDI(+)-MS.

Sorbitan esters (also known as SPANS) are also widely used as emulsifiers and stabilizers in food applications.[64] Recently, this additive has been used to structure vegetable oils in an attempt to develop gels with solid-like properties and reduced levels of saturated and *trans*-fats, since these fats may have negative effects on human health. Well-known and widely used SPANS include monolaurate (SPAN 40), monopalmitate (SPAN 60), monostearate (SPAN 80) and monooleate (SPAN 85). Mixtures of FA with different chain lengths are commonly used in the production of commercial sorbitan esters, resulting in a mixture containing different proportions of the mono-, di- and triester forms. The variability in the chemical composition of sorbitan esters can interfere in the fat crystallization process. In this case, EASI(+)-MS was used to qualitatively identify the compounds which are found in the commercial samples of sorbitan esters from different suppliers. The results showed that, despite being commercialized as sorbitan monolaurates, these samples actually contained sorbitan diesters with FA of different chain lengths, and their composition varied depending on the supplier.[65] EASI-MS was therefore proven to be a feasible and efficient tool to determine the composition of sorbitan esters and this technique may be useful in the quality control of emulsifiers and assurance in the industry.

3.3 Muscle products

The content of proteins, water and lipids is a key parameter when checking for spoilage of muscle products. Proteins are affected even by the freezing process, and they are very prone to many deterioration processes.[66,67] Mislabeling of fish and meat products is also a common illegal practice and a relevant task that may require DNA analysis.[68,69] Isotopic composition analysis has also offered some alternatives regarding the assessment of the authenticity and traceability of herds, revealing their feeding characteristics.[70] Lipids[71,72] may still be the alternative compounds that are able to solve many of these questions. Eberlin and co-authors have shown[73] that speciation for different fish and meats can be efficiently performed by TI-EASI-MS (Fig. 5). After rapid (few min) lipid extraction and analysis, beef, chicken, pork, mutton, sardine, trout and salmon were found to provide characteristic TAG signatures. For instance, the ion of *m*/*z* 827 assigned to $[PPPo/MPO + Na]^+$ was a major ion in the profile of TAG of beef samples, whereas the ion of *m*/*z* 975 assigned to $[S - EPA - DHA + Na]^+$ was a major ion in salmon samples. Multivariate analysis of the EASI (+)-MS data confirmed the ability of the technique to discriminate different types of meat and fish.

Eberlin and co-authors have also applied TI-EASI(+)-MS to access the traceability and authenticity of dry-cured hams.[74] Five types of dry-cured ham (Cebo, Recebo, Bellota, Serrano and Parma) were analyzed so as to acquire their profiles of FFA, DAG and TAG. The relative abundances of ions from oleic acid and also the molecules of DAG and TAG containing oleic acid could be directly related to the pig breed and feeding characteristics. Differences in ripening were also revealed according to the degree of TAG hydrolysis to DAG. As for the feeding characteristics, Bonafé *et al.*[75] also showed that the effect of the diet on the incorporation of conjugated linoleic and α-linolenic fatty acids into muscle tissue could be monitored by EASI(-MS). In this study, *Oreochromis niloticus* (Nile tilapia) was used as a model, and the profiles of FFA and chemometric analysis were used to group samples according to the type of diet employed for fish growth.

TI-EASI-MS has also been used to monitor degradation as a function of the conservation protocol and compositional changes from different storage conditions (*i.e.* time and temperature) in caviar samples of Atlantic sturgeon (*Acipenser sturio*), as shown in Fig. 6.[76] Freshly salted and commercially salted pasteurized caviar samples were stored for different periods and at different temperatures. Salted and pasteurized caviar showed different lipid profiles mainly related to the abundance of ions containing DHA (22:6) or EPA (20:5). Changes in the lipid profile from storage at room temperature and in the refrigerator could also be monitored as a function of the abundance of ions assigned as PC. Lipid oxidation was also verified and no oxidized lipids were found. Hydrolysis products were also detected, such as DAG and MAG. The thermal imprinting EASI-MS results were comparable to those obtained

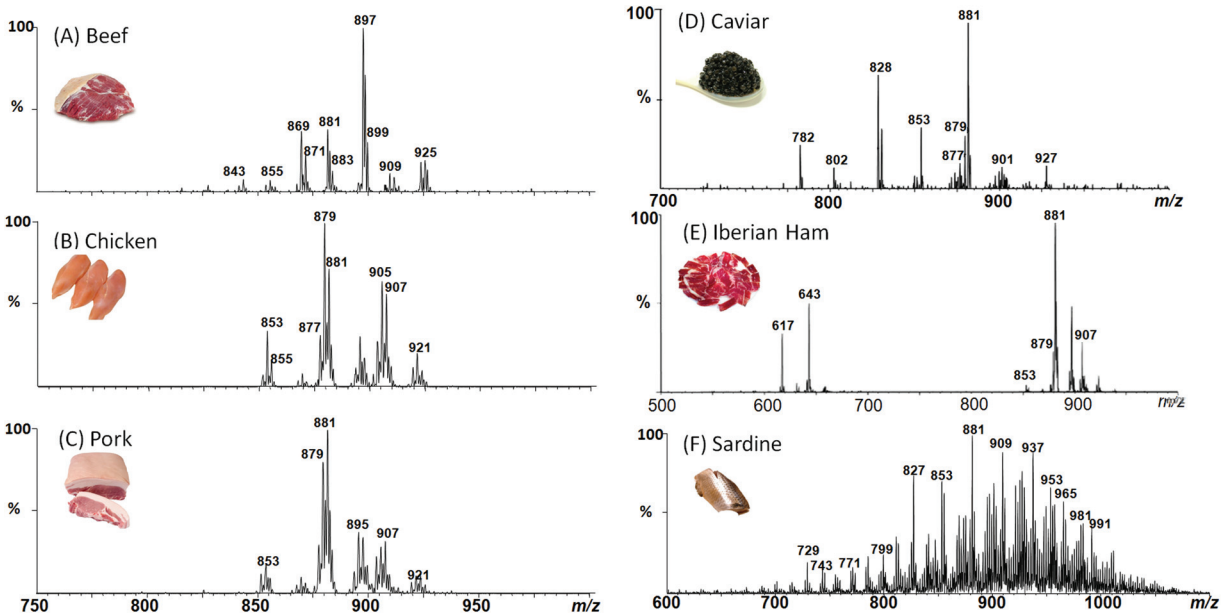

Fig. 5 TAG profile of diverse muscle products obtained by TI-EASI(+)-MS. (A) Beef, (B) chicken, (C) pork, (D) caviar, (E) Iberian ham and (F) sardine.

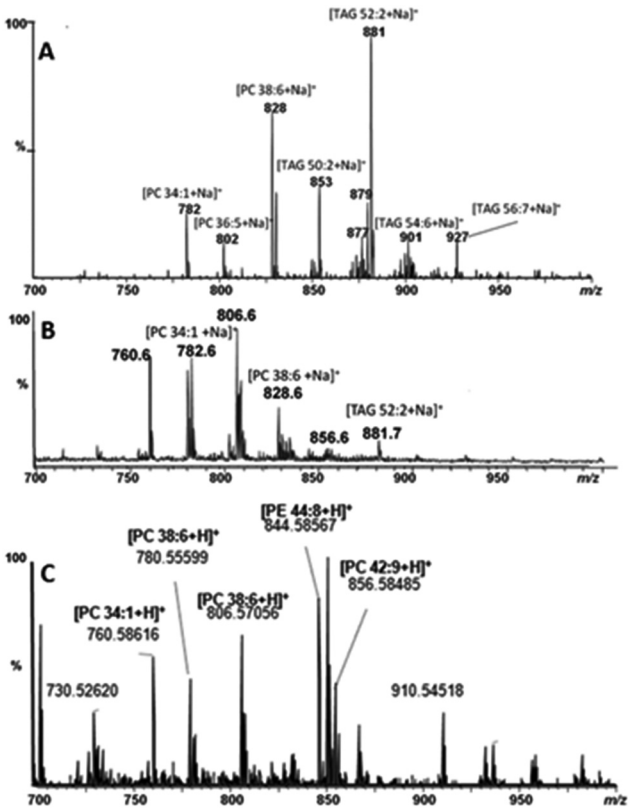

Fig. 6 Lipid extract of caviar samples analyzed by EASI-MS (A), MALDI-MS (B) and ESI-MS (C). In the EASI spectra, TAG and PL ions could be detected, as well as in the ESI spectra. In the MALDI data, PL are mainly detected and no information regarding the TAG ions is shown.

for the same sample after traditional Bligh & Dyer (BD) extraction.[41] TI-EASI was also compared to MALDI-MS for caviar lipidomics.[76] As observed for the lecithin analysis by both EASI-MS and MALDI(+)-MS, phospholipids such as PC were predominant in MALDI-MS, whereas both TAG and PC were detected by TI-EASI(+)-MS. This feature leads to a more comprehensive chemical characterization by TI-EASI(+)-MS, since the relative abundances of TAG and PL ions detected together can reveal additional information (Fig. 6A). As Fig. 6B shows, for MALDI-MS, the PL ions are so abundant that they suppress the ions corresponding to TAG ionization. The information provided by EASI-MS is, therefore, more similar to the one obtained by ESI-MS, as shown in Fig. 6C, but EASI still has the advantage of being an ambient technique.

3.4 Propolis

Propolis is a honey bee product with broad biological properties, such as antibacterial, antifungal, antioxidant, antiviral and anticancer properties.[77] The wide application of propolis has drawn growing attention towards the proper determination of its chemical composition and also to the correlation of such a composition to the listed properties.[78] Great variations in the composition and biological activities have been found for different types of propolis.[79–81] Propolis characterization is therefore extremely important for its standardization, practical applications and therapy. We have extensively evaluated the use of direct infusion ESI fingerprinting[82] for the direct, fast and reliable characterization of propolis samples from different origins, but we have also shown that such an analysis can be further simplified *via* EASI-MS.[83] For instance, a total of 49 samples of ethanol extracts of propolis collected worldwide (North and South America, Europe, Asia and Oceania)

were analyzed by EASI(−)-MS. Over half the samples analyzed had compounds derived from *Populus* resins as characteristic ions, thus indicating a clear preference for this plant genus by *A. mellifera* bees. Propolis derived from *Populus* resins displayed ions related to dihydroxyflavone and other well-known flavonoids, such as chrysin, pinocembrin, apigenin/galangin, pinobanksin, caffeic acid phenethyl ester, and pinobanksin acetate. In Mexico and Brazil, stingless bees frequently use resins from different plant sources in relation to *A. mellifera* bees. Propolis resins originating from these bees showed therefore a different composition. The EASI-MS data allowed the grouping of samples for similar plants and geographical origins, enabling the identification of the plant sources of propolis resins.

3.5 Coffee beans

Coffee is one of the most commercialized food products and the most widely consumed beverage in the world.[84] Coffee is also considered, a functional food because of its high content of antioxidants and other beneficial components. *Coffea arabica*, also known as Arabica coffee, is responsible for approximately 70% of the global coffee market, whereas *Coffea canephora*, or Robusta coffee, accounts for the remaining 30%. The post-harvest processes (dry, semi-dry or wet) greatly influence the coffee quality and cause variations in the body, acidity and flavor of the beverage. Recently, EASI-MS, and DESI-MS were applied directly to the surface of intact green Arabica coffee beans. Both techniques were found to function as fast and simple protocols to differentiate beans treated by the dry, semi-dry and wet post-harvest methods.[85] Five coffee phytomarkers were monitored and three of them (β*N*-arachinoyl-5-hydroxytryptamide, β*N*-behenoyl-5-hydroxytryptamide and β*N*-lignoceroyl-5-hydroxytryptamide) were identified as components of the wax layer that covers the coffee bean, and these compounds are generally associated with stomach irritation in sensitive persons who consume coffee drinks.[84] Using PCA, it was indeed possible to follow the differences among the coffee beans according to the post-harvest treatments.

Recently, since the power of deep penetration into the surface is naturally limited for both the spray based techniques, DESI and EASI, as well as for DART which is based on "ionizing He gas", an alternative methodology has been proposed. This methodology was found to enable more efficient biomarker desorption from intact coffee beans.[86] This new approach has been term Venturi-assisted laser desorption, that is, VALDI (Fig. 7). The proposed system was able to analyze samples placed far from an ESI source, and it was demonstrated to be easily connected to commercially available API sources. In this study, ESI was used for the ionization process; however, the coupling of the VALD interface to SSI is also possible and is under testing in our laboratory.

3.6 Food contamination by pesticides

The use of ambient MS techniques to monitor pesticides in food products seems quite promising. An indication of such applicability has been presented for glyphosate (*N*-phosphonomethyl glycine), which is the world's most widely produced herbicide, by volume. This compound has been extensively used and its consumption has increased in agriculture, thus being a risk for both human and environmental health.[87] We[88] analyzed a solution of glyphosate (50 μg mL^{-1}, MeOH, 0.1% NH_4OH) by S-EASI(−)-MS and observed an abundant and predominant presence of the $[M - H]^-$ ion of *m*/*z* 168. Such an S-EASI source could therefore be tested for the direct monitoring of pesticides on crop plants, food products and beverages in general, particularly for drinking water, and such a study is underway in our laboratory.

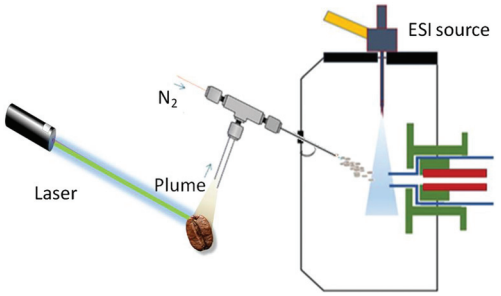

Fig. 7 Scheme of the direct Venturi-assisted laser desorption interface coupled to an ESI source as applied to a coffee bean. Adapted from ref. 86.

3.7 Beverages

The characterization of several beverages, such as whiskey, wines, *cachaça*, rum and yerba mate extracts have been performed *via* direct infusion ESI-MS analysis, focusing on authenticity, counterfeiting and typification. For instance, whiskey[89] counterfeiting has been monitored using a pool of typical wood phytomarkers detected only in authentic whiskeys. Brazilian *cachaça*[90] samples aged in different wood barrels were also efficiently monitored, and fraud through artificial aging could be properly detected. Although such studies have focused on the direct infusion ESI-MS analysis, the possibility to further simplify such an analysis *via* ambient MS seems certain and it has already been demonstrated using liquid V_L-EASI,[36] as well as the S-EASI[88] technique.

4. Quantification

MS techniques using ambient ionization have been widely applied in qualitative analysis but little has been done in relation to quantification. Perhaps, the main problem related to these techniques in regard to quantification concerns their low repeatability/reproducibility. Although there is still much to be tested to fulfill the extensive list of experiments required for the validation of quantification methods, some studies have reported acceptable figures of merit, such as limit of detection (LOD), dynamic range and linearity for ambient MS. The use of internal standards (IS), preferably isotopologues,

carefully homogenized throughout the sample seems to be the best approach for superior quantification by ambient MS.[91] This procedure has however the disadvantage of disturbing the spatial resolution of the analyte. The use of IS may also introduce additional sample preparation steps, which are therefore not desirable for an ambient MS technique.

But one should always consider that the main purpose when applying EASI-MS in food analysis is not to perform a comprehensive composition analysis or precise quantification but to mostly provide data on typification and quality control *via* characteristic chemical signatures. But EASI-MS has also been shown to provide proper quantification, as demonstrated by the determination of the composition of TAG of edible vegetable oils, hydrogenated vegetable fats and cocoa butter.[16] Results were compared with those obtained by GC-FID and also by theoretical predictions of the composition of TAG performed by a software projection after GC-FID detection. Acceptable correlation coefficients were observed between the three methods during the analysis of vegetable oils and hydrogenated vegetable fats. The reproducibility of the quantitative results was *ca.* 10–15%. EASI(+)-MS seems to offer not only an appropriate qualitative tool for oil analysis but also a precise and detailed way to perform quantification. The profiles of FFA obtained by EASI(−)-MS can also be used to access the acidity of vegetable oils, since FFA can be quantified, using internal standards, with reasonable linearity ($r = 0.98$).[14]

5. Conclusions

The recent introduction of ambient desorption/ionization MS techniques has offered a variety of new tools in food analysis. Such techniques are simple, easy to perform and robust, requiring little to no sample preparation. High-throughput analysis can be performed providing both qualitative and quantitative information about major and minor components in various food samples. EASI-MS, because of its unique combination of figures of merits, including superior simplicity, bipolar ion production, voltage-free environment and superior S/N as well as absolute ion abundances, seems to be quite attractive for food analysis. After a decade of investigations, several applications of EASI(±)-MS to food analysis have been demonstrated and their main findings have been summarized in this review. The unique and detailed profiles of the marker components produced by rapid EASI(±)-MS screening provide a quite comprehensive monitoring of food authenticity and quality. The use of such a simple and robust technique may also facilitate on-site food screening. This application is illustrated by the recently described S-EASI-MS technique, using the most simplified design of an EASI source, which can be most conveniently coupled to compact/portable mass spectrometers. Such a monitoring could, for instance, be performed by control agencies directly on production sites or provide fast analysis in the laboratory in the cases of contamination, fraud or authenticity check. The quite universal application of such techniques also allows their use in a myriad of different food products. A new, rapid, simple and quite comprehensive and efficient tool is now available for food analysis at the molecular level.

Abbreviations

EASI	Easy ambient sonic-spray ionization
MS	Mass spectrometry
ESI	Electrospray ionization
DESI	Desorption electrospray ionization
DART	Direct analysis in real time
TAG	Triacylglycerols
FFA	Free fatty acid
S/N	Signal/noise
PL	Phospholipids
PC	Phosphatidylcholines
PE	Phosphatidylethanolamine
LPL	Lysophospholipids
PCA	Principal component analysis
P	Palmitic acid
O	Oleic acid
L	Linoleic acid
Ln	Linolenic acid
La	Lauric acid
Ca	Capric acid
M	Myristic acid
IS	Internal standard
Po	Palmitoleic acid
DHA	Docosa-hexaenoic-acid
EPA	Eicosapentaenoic acid

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