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REVIEW

Mass Spectrometry Imaging for Vegetables: A Review

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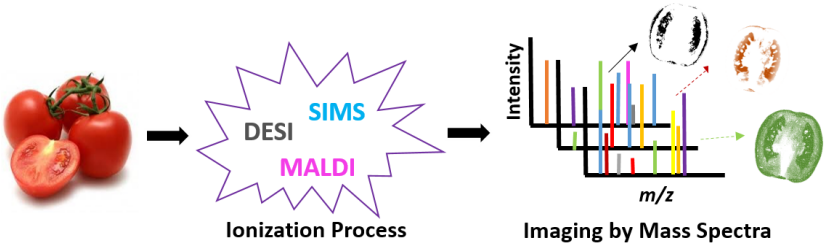

In this review, the use of mass spectrometry imaging (MSI) was addressed, focusing on the study of plant tissues, especially vegetables. The discussion about the taxonomy of plant tissues and organs is commonly based on immunohistochemistry and immunofluorescence essays. Although these techniques are quite appropriate for the structural study of tissues and organs, their low specificity limits their use to the identification of only a few compounds. Mass spectrometry (MS) hyphenated with chromatography techniques are capable to identifying a wide variety of compounds in plant tissue matrices, but these analyzes do not provide spatial information of the sample. MSI techniques stands out in this scenario due their capacity to provide information about both composition and spatial distribution of different biological matrices in the in the same approach. The potential of the MSI techniques to provide information about primary and secondary metabolites in plant tissue, as well as chemical responses associated with external stimuli, can be demonstrated through published works that employ different ionization sources such as SIMS, MALDI and DESI and their modifications.

Keywords: mass spectrometry imaging, ambient ionization methods, food analysis, mass spectrometry, plant tissue analysis

INTRODUCTION

Vegetables play an essential role in human nutrition and health by providing nutrient bioactive molecules (e.g., vitamins, minerals and dietary fiber) and non-nutrient phytochemical compounds (e.g., flavonoids, phenolic compounds, bioactive peptides, etc.). These nutrient and non-nutrient compounds contribute to the control and prevention of cardiovascular diseases, diabetes, cancer and metabolic syndromes, among other health problems.[1]

Like any other plant, vegetables are sessile organisms, and cannot escape their environment. Evolutionarily, this condition resulted in a complex metabolism with over 200,000 known primary and secondary metabolites. These compounds are generated from specific metabolic adjustments that the plant is able to promote in response to external stimuli.[2] The primary metabolites are directly involved in plant growth, development, and reproduction (e.g., carbohydrates, amino acids, fatty acids, and organic acids, etc.), while secondary metabolites are defense compounds produced by plants as a result

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of environmental interaction or environmental stress (e,g., terpenoids, phenolics, alkaloids, and sulfur-containing compounds such as glucosinolates, etc).[1,2]

In the literature, the meaning of the word "vegetable" is broad and may vary according to biological and cultural factors. The biological definition states that vegetables are any part of a plant consumed as food, except for mature fruits or seeds. For example, this includes tubers such as white potato (*Solanum tuberosum*), bulbs such as onion (*Allium cepa*), roots such as carrot (*Dacus carota*), entire leaves such as lettuce (*Lactuca sativa L.*), petioles such as celery (*Apium graveolens*) and immature fruits such as cucumber (*Cucumis sativus*). However, culinary definitions based on the cultural use of some foods classify mellow fruits such as tomato (*Solanum lycopersicum*) as vegetables. This controversial classification is based on the use of mature fruits as part of a main meal, rather than dessert. Surprisingly, it has already been decided by the US Supreme Court Nix v. Hedden (1893) that tomatoes should be classified as a vegetable.[3]

Although other seeds and mature fruits are colloquially associated with vegetables due to anatomical similarities and mode of consumption, the biological diversity among vegetables requires the systematic methods for grouping and classifying to support management decisions associated with production, storage, transport and marketing of these crops.[1,3] There are specific characteristics that are employed to classify vegetables based on their tissues and organs consumed as food, as well as in their taxonomy and ecological adaptation (e.g., proper temperature and amount of light for cultivation). Table I presents the correct classifications of some polemics vegetables commonly consumed in various culinary cultures around the world.[3,4]

Table I. Botanical names, common names and classification of some vegetables

Botanical name	Common name	Vegetable classification
Abelmoschus esculentus L. Moench.	Okra, gumbo	Fruit
Solanum lycopersicum Mill.	Tomato	Fruit
Raphanus sativus L.	Radish	Root
Brassica oleracea L.	Cabbage	Leaf
Cucurbita argyrosperma Huber	Pumpkin	Fruit
Brassica oleracea L. Italica group Plenck	Broccoli	Flower
Capsicum annuum L. Grossum group	Bell pepper	Fruit

The discussion about the correct classification of plant tissues and organs is commonly based on immunohistochemistry and immunofluorescence techniques, that use optical and electron microscopy to provide structural information of biological tissues via images. These analytical tools are the most used to differentiate and classify tissues and plant organs. Although these techniques are quite appropriate for taxonomic studies, the low specificity limits their used to the identification of only a few compounds, such as proteins and genetic material.[5] The identification of the metabolic present in plant tissues can also be based on Mass spectrometry (MS) technique hyphenated with separated techniques such as gas and liquid chromatography. This technique requires the use of proper extraction methods for sample preparation and, despite being capable of identifying a wide variety of compounds within a broad concentration range, their use of this methodology results in loss of spatial information.[6]

The capability of analytical techniques in providing information about both composition and spatial distribution of different biological matrices has improved in the last two decades, and mass spectrometry imaging (MSI) has stood out in this scenario.[7,8] The basic concept of the MSI technique can be summarized in four steps: sample selection and preparation, desorption/ionization, mass analysis and image registration

and data analysis (Figure 1). First, sample pretreatment is generally based on fast frozen of the sample for subsequent sectioning into thin slices with a cryostat. To desorb/ionize analytes in the sample, each slice is fixed onto an appropriate target plate by thawmounting. A computer-controlled sample stage can then be used to promote desorption/ionization spot-by-spot over a defined sample area. The ions from each spot are separated by the mass spectrometer analyzer and a spectrum of each spot is generated. Combining the data obtained over the entire sample area, one can plot the intensities of individual mass-to-charge ratio (*m/z*) peaks against the x-y coordinates. This will generate two- or even three-dimensional chemical images corresponding to the spatial distribution of the selected ratio (*m/z*), which can be associated with the original histological features of the sample.[9,10]

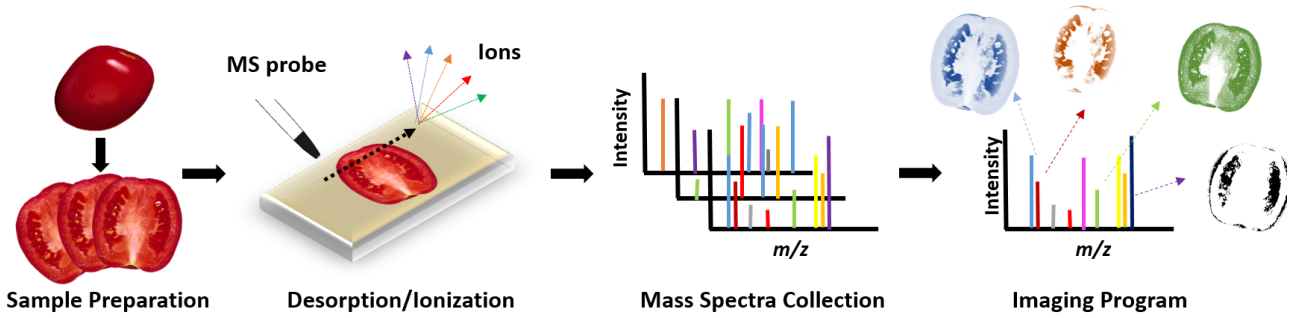

Figure 1. Main steps involved in using the MSI technique: sample preparation; desorption/ionization; mass collection and imaging program.

Over the years, the number of papers about MSI has increased, but most of them focus on the imaging of animal tissue only. Due to some technical barriers, such as difficulties in sample pretreatment caused by morphological diversity, plant tissues are still little explored by the MSI technique. To demonstrate the potential of the MSI technique for plant tissue analysis and encourage the development of research that contribute to the description and classification of vegetables, the present review refers to a set of papers found by using the Google Scholar® platform. It is important to emphasize that, despite being free, this platform does not only list articles in indexed journals. For this reason, all articles cited here have been checked to ensure they have been peer-reviewed.

The search equation was built on the platform by adding the terms “plants” or “fruits” and or “vegetables” in the sentence “mass spectrometry imaging”, focusing only on article titles. This strategy was used due to the broad definition of the word “vegetable”, as discussed above. The time-lapse considered during the search was between the years 2012 and 2021. The search results are presented graphically in Figure 2.

The number of published articles containing the expression “mass spectrometry imaging” in the title has gradually increased in recent years within the platform. However, among these articles, those containing the additional words “vegetable”, “plant” or “fruit” in the title are less than 5%. Despite the growth trend observed in the graph, it is clear that the study of vegetables and other plants is a field still underexplored by mass spectrometry imaging.

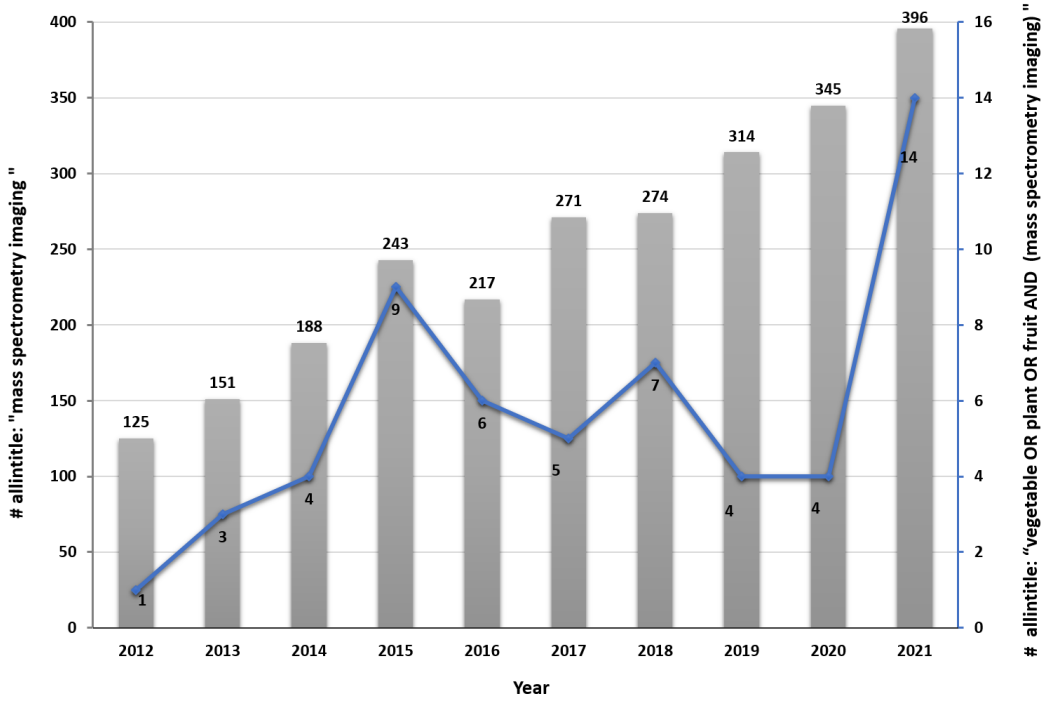

Figure 2. Publication trend (number of published articles) related to search equations "mass spectrometry imaging" (grey bars) and "vegetable OR plant OR fruit AND (mass spectrometry imaging)" (blue line). The number of publications were determined by using Google Scholar for research articles published from 2012–2021 containing the cited words in the title.

In sequence, we briefly describe the principles and applications of MSI technique for vegetables analysis and some important considerations for planning and executing experiments.

MSI IONIZATION METHODS

The main difference between the MSI techniques employed to analyze plant tissue is the way ions are generated. Among the most used ionization methods for this purpose are Secondary Ion Mass Spectrometry (SIMS), (Matrix-Assisted) Laser Desorption Ionization (MALDI/LDI) and Desorption Electrospray Ionization (DESI).[11,12] Modifications of these techniques have also been investigated in order to adapt them to the different characteristics of biological matrices.

Secondary Ion Mass Spectrometry Imaging (SIMS)

SIMS was the first MSI method developed to image analytes on the surface of samples (Yoshimara 2021). It operates under high vacuum and uses high energetic primary ions (e.g., Ar^+, Ga^+, Bi^+, Bi^{3+}, C^{60+}, Au^+, Au^{n+}, Ar^{n+}, and Cs^{n+}) for ionization (5 – 40 keV). The advantage of this technique over the others is the very high spatial resolution (better than 100 nm) obtained due to the focused primary ion beam. When the primary ions collide with molecules in the sample surface, they transfer their charge to the sample molecules generating secondary ions.[2,5,10] This "harder" ionization process causes fragmentation and makes the identification of the molecular ion more difficult. Therefore, its use for imaging of plant material is limited and SIMS ends up restricted to elemental ions and molecules whose fragmentation pattern is easily recognized (e.g., lipids, fatty acids and triglycerides).[13,14]

Matrix-Assisted Laser Desorption Ionization (MALDI)

MALDI is widely used as a soft ionization method for analysis of biological tissues by MS. This technique employs laser (UV or IR) for the direct desorption/ionization of compounds present on the surface of a

sample that has been co-crystallized with an organic matrix. The exact mechanism of the desorption/ionization in MALDI is not fully understood, but it is well known that utilization of matrix efficiently intensifies the ionization process so that lower laser intensities are required, which minimizes fragmentation. Typically, the matrix is composed of small organic acids, such as α-cyano-4-hydroxycinnamic acid (CHCA – 189 Da) and/or 2,5-dihydroxybenzoic acid (DHB – 154 Da), that are deposited on the sample surface by a sprayer or by solvent-free sublimation.[15,16] For compounds that are sensitive to acids or ionize better in negative mode, alternative matrices such as 9-aminoacridine (9-AA) are more suitable.[4,5] Although matrices prevent fragmentation of analytes, they also create drawbacks. First, matrix background signals easily appear in the low mass range and hinder the analysis of small molecules. Second, the size of matrix crystals limits the spatial resolution of images (5 – 200 µm). Due to these limitations, novel matrices and matrix deposition methods are the subjects of study of many recent researches in MALDI-MSI.[9,17]

To reduce the interference of matrix in the low mass range, other matrix-free ionization processes – e.g., Laser Desorption Ionization (LDI) – can be adopted. Recently, LDI-MSI with gold nanoparticle-enhanced target (AuNPET) was used for visualization of small molecules (e.g., anthraquinone derivatives and their glucosides, stilbenes, anthocyanins, flavonoids, polyphenols, organic acids, chromenes, chromanones, chromone glycosides and vitamins) in rhubarb stalk (*Rheum rhabarbarum L.*), considered a medical plant and a vegetable in Chinese culture.[18] LDI-MSI with silver nanoparticles (AgNP) was also employed to identified the distribution of low molecular weight metabolites belonging to aldehydes, ketones, alcohols, esters, organic acids, phenolics, amino acids and sugars classes present in strawberry fruit cross-section.[19]

Desorption Electrospray Ionization (DESI)

In desorption electrospray ionization (DESI) charged solvent microdroplets are launched via the ESI source against the surface of the sample. The microdroplets transfer charge to the analytes present on the surface, promoting their desorption from the sample. The advantage of DESI is the direct ionization under atmospheric conditions without demanding complex sample pretreatment. DESI has been used to ionize a wide range of compounds, however, such ionization has limitations in terms of spatial resolution, usually 50 – 500 µm.[20] This is due to the difficulty of producing a convergent and well-controlled solvent spray angle, as it is possible with a laser (MALDI) or ion beams (SIMS). The most suitable solvents for DESI should have low surface tension, such as methanol and acetonitrile. Thus, DESI is not suitable for ionizing non-polar substances. Due to the simple sample preparation required, DESI is suitable for the analysis of sensitive plant tissues, leaves, flower petals and fruit peel.[2,21]

Other ambient ionization techniques have also been used to image plant tissues.[8] Laser ablation direct analysis in real time imaging-mass spectrometry (LADI-MS) uses lasers to ionize the analytes under atmospheric pressure, a kind of combination between MALDI and DESI.[22] A technique that uses a Low Temperature Plasma (LTP) composed of excited helium gas was also used to ionize analytes in vegetables under atmospheric conditions. The LTP is capable of ionizing a wide range of low-molecular weight compounds, including low polarity substances. Laser Desorption (LD) that uses continuous ultraviolet wave (UV) diode laser was also associated to LTP probe to analysis. It has been used to detect mescaline on the cross-section of *Echinopsis pachanoi* cactus and other plant matrices for forensic purposes.[23]

Spatial Resolution is undoubtedly a very important issue that must be taken into account when analyzing metabolites in plant tissues.[11] Unfortunately, high spatial resolution in MSI results in loss of sensitivity. TOF-SIMS can produce the highest spatial resolution in submicron level when compared to the other cited ionization techniques. As for DESI, despite not producing fragments in the ionization process, its spatial resolution can only reach μm, as it depends on the amplitude of the solvent droplet spray. The spatial resolution of commercial MALDI MSI instruments is also in the µm range and is mainly determined by the size of the laser spot and the matrix itself. Thus, some studies to improve the spatial resolution of MALDI-MSI focusing on laser spot reduction have been conducted.[2] Another key point that must be considered in the experimental design is the size of the sample to be imaged. Very large samples can take up to hours of analysis to obtain a good spatial resolution. Table II presents the most important characteristics of the ionization techniques that must be considered to plan the analysis of plant tissue.

Table II. Comparison of ionization methods used for MS imaging (MSI) analysis of plant tissue samples

	SIMS	MALDI	DESI
Probe beam	Ion	Laser	Solvent
Spatial resolution	50 nm – 5 μm	5 – 200 μm	50 – 500 μm
Pressure	Vacuum	Vacuum	Ambient
Target Molecules	Elements, fatty acids, lipids	Metabolites, lipids, peptides, carbohydrates	Lipids, peptides, small metabolites
Advantages	High spatial Resolution	Cover a wide mass range	Simple sample pretreatment
Drawbacks	Analytes prone to fragmentation at source	Matrix background signals	Unfavorable for non-polar substances

SAMPLE PREPARATION TECHNIQUES

Sample preparation steps for analysis of vegetables (or any plant tissue) vary depending on the MSI instrument employed as ionization source, on the structural nature of the sample and on the analytes to be imaged. To discuss sample preparation comprehensively, the most important steps including tissue storage, sectioning, mounting and others sample pretreatment will be briefly discussed below.

Storage

For storage, samples are generally flash frozen in liquid nitrogen and then stored at -80 ºC. The rapid freezing is a key action to minimize enzymatic degradation and analyte migration within the biological matrix. Attention should be paid to water-rich vegetable samples, since long-term storage can cause structural changes due to the gradual loss of moisture. When the sample has a large thickness and a three-dimensional analysis is desirable, vegetable samples can be stored as section slides.[4,10,24]

Sectioning

Plant cells have rigid walls and large intercellular spaces filled with water. Thus, in order to maintain the morphology of the tissue, it is common to use embedding materials to ensure a reliable cut of the sample in conventional plant histology practice. The embedding process can be carried out in two ways: the sample can be frozen either before or after being added to the embedding material. In both cases an adhesive film can be attached to the embedded sample to help providing an accurate cutting and slicing of the sample. Unfortunately, the use of this sample preparation methodology is limited, since many materials used for embedding the sample are incompatible with the MSI. For example, ideal temperature cutting compound (OCT), which is a blend of polyethylene glycols, should be avoided as it can diffuse to the surface of the sample.[25] Carboxymethylcellulose (CMC), gelatin, ice or their combinations have been successfully employed as MSI-compatible embedding mediums. It is important to notice that, when using materials for this purpose, care must be taken to avoid mischaracterization of the sample.[26-28]

Cryosection is the most commonly used method to prepare slices of plant tissue. Samples can be frozen in different conditions for later cutting into slices. The use of ultra-low freezer temperature, dry ice powder and liquid nitrogen are some of these conditions. Section thickness is another parameter that needs to be controlled. The ion intensity signals in MSI instruments can be altered due to the thickness of the analyzed section, which can generate divergent results that do not correspond to the intrinsic characteristics of the sample.[29,30]

Mounting

Mounting the sample tissues for analysis can be done on a glass slide or on a MSI compatible platform. Many different surfaces can be employed depending on the ionization technique. For non-orthogonal MALDI-TOF, conductive surfaces such as metal coated glass slices are required for application of the electrical potential that will orient the ions. As for the orthogonal MALDI-TOF, common sheets can be used. Atmospheric pressure ionization techniques such as DESI do not need a conductive surface during the ionization process either.[10,28]

Thawed assembly is another way to mount sections of tissue acquired by cryosectioning. This approach avoids sample contamination, but migration of water-soluble analytes during thawing can mischaracterize the sample. Such effect was discussed in the work of Kim *et al.*,[14] in which two sample preparation methods were used for the analysis of corn seeds by SIMS-MSI (Figure 3). Unlike thaw-mounted sample preparation, the method that used an adhesive tape during cutting and fixing of the sample does not cause imaging distortion due to the migration of the analytes.

Other possibilities

Depending on specific characteristics of the samples, the sample preparation process for MSI analysis can be simplified. Fresh plants, for example, can be analyzed by MSI employing simple sample preparation methods or even without sample preparation at all. This can be done by using ambient ionization techniques such as DESI, avoiding chemical and morphological changes resulting from sample preparation.[21,31] However, it must be accounted for that biological processes are still active in fresh tissue, which can lead to degradation and/or chemical changes during the analysis. An alternative is the analysis of dehydrated samples. For instance, the spatial distribution of curcumin in dried turmeric root was examined by MALDI-MSI without embedding for sectioning.[32]

Land plant organs, such as leaves and flowers, have a lyophilic cuticular layer (0.1 – 10 µm thick) composed of lipids, which provides protection, among other biological functions. Unfortunately, this cuticle is a barrier to MS imaging of metabolites present in inner layers when soft ionization techniques such as DESI and MALDI are used. The cuticle can be scraped off or chemically removed with organic solvents. Nonetheless, “aggressive methods” can displace and/or remove surface compounds of interest.[10,33]

The inprinting method is an alternative approach in which plant tissues are pressed onto porous Teflon, TLC plate, print paper or tape by applying light pressure on plant tissues. Thus, plant metabolites and others substances present in the sample are transferred to the flat surface, conserving their space distribution.[14,20,34]

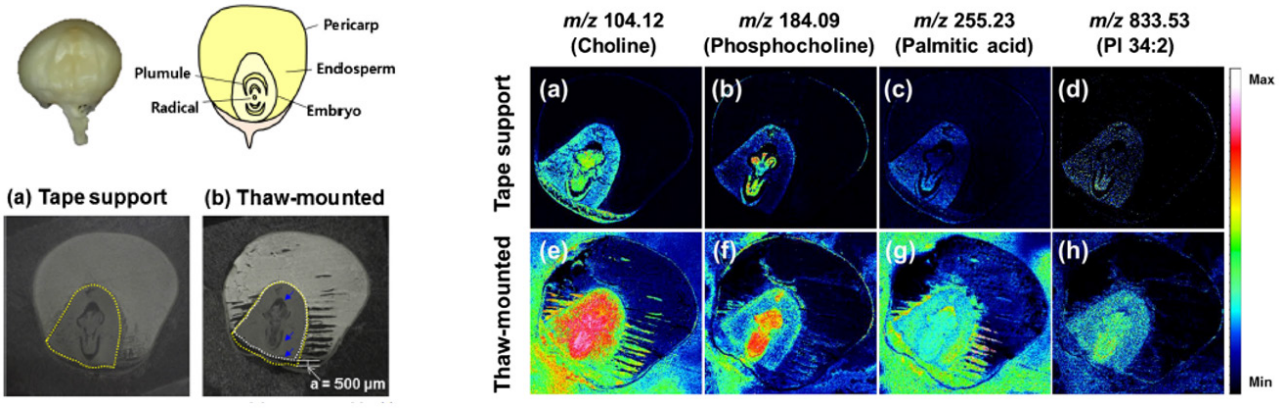

Figure 3. Optical images of corn seed tissue prepared by tape support and thaw-mounted methods; SIMS images of corn seed tissue prepared by the two methods demonstrate differences in ion distributions. [Reprinted with permission from Kim, S. H.; Kim, J.; Lee, Y. J.; Lee, T. G.; Yoon, S. Sample Preparation of Corn Seed Tissue to Prevent Analyte Relocations for Mass Spectrometry Imaging. *J. Am. Soc. Mass Spectrom*. 2017, 28 (8), 1729 – 1732. Copyright 2017. American Chemical Society.]

APPLICATIONS OF MSI ANALYSIS FOR VEGETABLES

The versatility of the MSI technique for analysis of vegetables can be easily demonstrated through recently published papers. Different tissues of plant organs consumed as food, i.e., fruits, roots and seeds were already characterized via MSI as a function of the spatial distribution of primary and secondary metabolites.

Three-dimensional MALDI-MSI was used to produce the first 3D image of wheat grain, representing the structural variations of primary metabolites Arabinoxylans (AX) and β-glucans (BG) across the endosperm from the brush to the germ of the grain. These polymers and their spatial distribution can impact the water content of the grain, which can affect its final quality.[35] The same technique has already been applied to monitor metabolic processes during the germination of maize seeds during different time intervals after imbibition – the first stage of seed germination, which consists of the absorption of water by the seed cells. The identified metabolites include sugars (monosaccharides and disaccharides), sugar acids, organic acids, phenolics, nitrogenous metabolites, polyols, esters, lipids, fatty acids and sterols.[36]

To investigate changes in spatial distribution of lipids and other small molecules at a cellular level in response to salt stress, MALDI- MSI and other complementary techniques were used to analyze seeds of two barley genotypes with contrasting germination phenology (Australian barley varieties Mundah and Keel). Seed germination is the essential step in crop process, and can be severely affected by ambient stress, such as salinity. Salt stress can inhibit essential metabolic processes during the germination and trigger lipid-dependent signaling reaction that activate plant adaptation processes.[37] Lipids present in thylakoid membranes of maize leaves have also been investigated by MALDI-MSI. These membranes are the universal site of the photochemical and electron transport reactions of oxygenic photosynthesis in cyanobacteria and plant chloroplasts.[33]

MALDI was also applied to visualize the spatial localization of the main steroidal alkaloids in diverse plant tissues of *Lycopersicon esculentum*, *Solanum nigrum*, and *Solanum dulcamara*. In addition, multivariate unsupervised principal compound analysis (PCA) and the k-means clustering analysis were calculated to characterization of the tissues and organs with respect to their chemical similarity. Alkaloids are common secondary metabolites found in plants of the large family of *Solanaceae*, commonly known as the nightshades, which includes important crops, such as potatoes (*Solanum tuberosum*), tomatoes (*Solanum lycopersicum*), eggplants (*Solanum melongena*) and peppers (*Capsicum species*).[38]

Furthermore, MALDI-MSI was employed to visualize the spatial distribution of the endogenous molecules during wolfberry fruit development, a traditional herbal medicine in Asian countries. From the mass spectrum imaging, the choline, betaine and citric acid were observed throughout the entire fruit at all development stages. The hexose was distributed in the endocarp and flesh tissue, while sucrose was located in the seeds. Additionally, several phenolic acids and flavonoids were accumulated in the exocarp during fruit development, which indicated that they seemingly played protective roles in wolfberry fruit growth progress against abiotic and biotic stress.[39]

Techniques similar to MALDI have also been used to provide images of vegetables. Infrared Matrix-Assisted Laser Desorption Electrospray Ionization (IR-MALDESI), in turn, has already been used to obtain the metabolic profile of cherry tomatoes. This MSI technique operates at ambient conditions and simple sample pretreatment based on flash frozen and cryosection is required. The metabolic profiling identified diverse array of metabolites including amino acids and lipids along with the major secondary metabolite classes: terpenes, phenolics, glycosides, and alkaloids.[40] LDI-MS was employed to monitoring pesticide penetration into the interior of various vegetables (*i.e.*, apple, cucumber, pepper, plum, carrot, and strawberry). A filter paper containing golden nanoparticles (AuNP) was used in a simple sample pretreatment to imprinting vegetable tissues. The use of nanoparticles improve ionization efficiency, overcoming the deficiencies of conventional imprinting materials (Figure 4).[41]

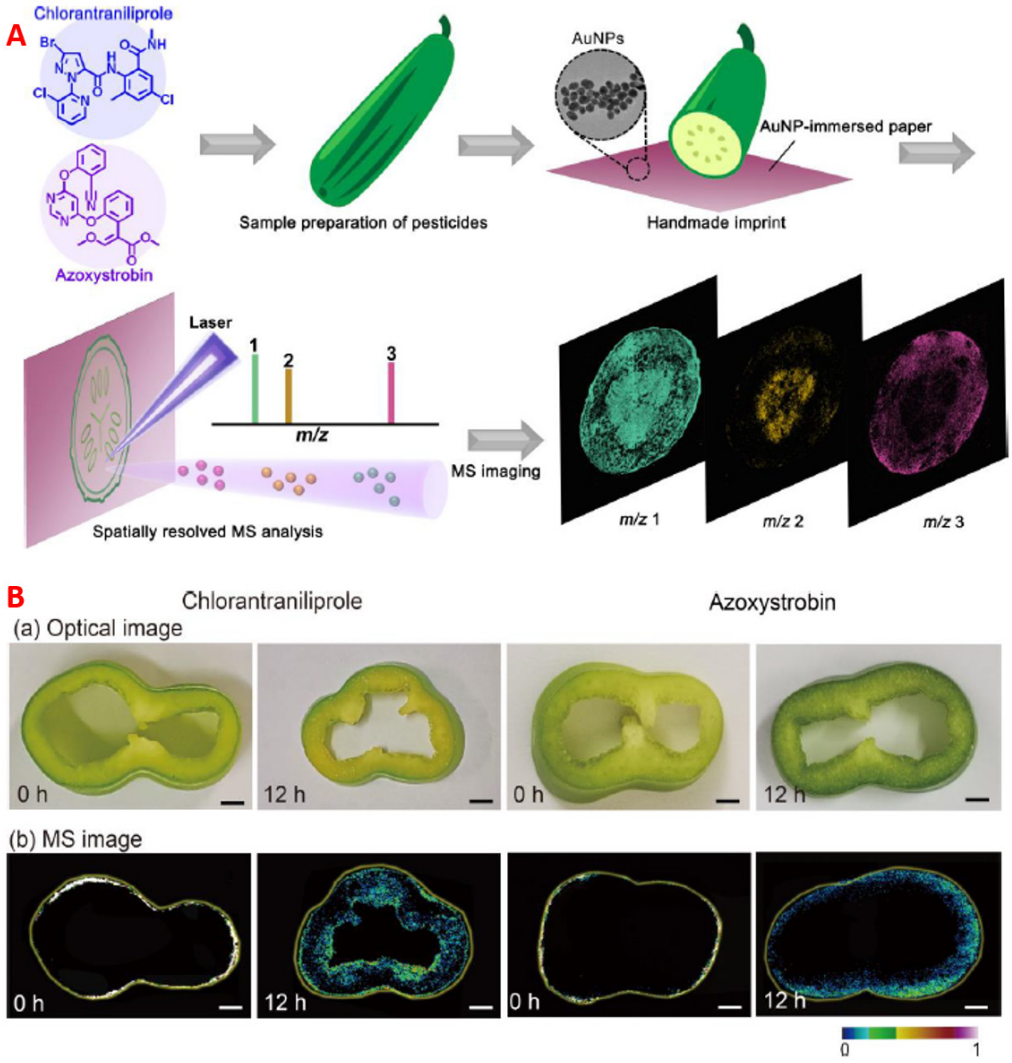

Figure 4. (A) AuNP-immersed paper imprinting scheme for LDI-MSI analysis of different vegetables. (B) Optical images and MS images of pepper slices 0 h and 12 h after the application of pesticides chlorantraniliprole and azoxystrobin. Images adapted from the reference N.º 41, which is an open access article distributed under the Creative Commons Attribution License.

Conventional techniques involving chromatography can also be associated with MSI analyzes of vegetables to confirm the results. For example, DESI-MSI was used to visualize the plant hormone abscisic acid (ABA) and the jasmonic acid related-compound 12-oxo-phytodienoic acid (OPDA) in immature (*Phaseolus vulgaris L.)* seed sections. These compounds play crucial roles in seed development, dormancy, and germination. In addition, the localization of ABA and OPDA using DESI-MSI was confirmed using liquid chromatography-MS/MS (LC-MS/MS).[42]

CONCLUSIONS AND FUTURE PROSPECTS

Among the techniques presented in this review, MALDI stands out as the most widespread one. Despite having some limitations regarding the use of matrices that help the ionization process, such as matrix effects, their high resolution associated with the analysis of molecules with a wide range of *m/z* ratios justify their broad use. New matrices have been developed to minimize matrix effects in MALDI analyses and, recently, NPs based on metals and silicon have been used as a matrix instead of conventional organic

matrices.[18,19] Ambient ionization techniques have increasingly attracted the attention of researchers who employ mass spectrometry to a wide variety of matrices.

DESI and its modifications require relatively simple sample preparation steps. However, its high detection limit is a disadvantage. For this reason, modifications aimed at improving the DESI spatial resolution have been developed. Although being already well discussed in the literature, nanospray desorption electrospray ionization (nano-DESI) is an ambient technique that enables sensitive imaging of fully hydrated biological materials with high spatial resolution. However, the technique is still not widely used for the analysis of plant tissues. Nano-DESI utilizes localized desorption of analyte molecules from surfaces into a liquid bridge between two fused silica capillaries, followed by nanospray ionization of the molecules.[43]

In addition to issues associated with improved spatial resolution provided by ionization techniques, there are also challenges related to sample preparation methods. This stage of the analysis must guarantee the "stopping" of the metabolic process without altering the tissue structure. For this reason, several preparation methods have been developed and adapted to suit the most different types of plant tissues.

In short, there is no doubt there is a universe to be explored when we think about the use of MSI techniques to analyze plant tissues, especially those consumed as food such as vegetables. However, when compared to animal tissue studies, MSI still has a vast field of action for plant analysis. Primary and secondary metabolites associated with plant development, maturation and reproduction can be reliably investigated *in situ*. In addition, ambient conditions that stress and modify the metabolomics of plant beings can be monitored and investigated through MSI analyses.

There are recently published works in the literature that discuss whether or not plants can have consciousness. Of course, it is not about a human-like consciousness, but rather related to how these beings react to external stimuli, as well as to limited conditions of survival that stimulate competition between species. Although the topic has a somewhat philosophical character, MSI techniques are a great tool to support the discussion in a scientific way.[44,45]

Conflicts of interest

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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