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Review

Analytical capabilities of mass spectrometry imaging and its potential applications in food science

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ABSTRACT

Background: Mass spectrometry imaging (MSI) is an untargeted and label-free chemical imaging technique that allows for the analysis of hundreds to thousands of molecules in a single experiment. Over the last two decades, MSI has become common in the medicinal, pharmaceutical, and botanical research communities, but has been applied less frequently in food science research. As an emerging "molecular microscope", MSI offers unparalleled advantages for exploration of the spatio-chemical information from various food materials. It allows researchers to localize biomarkers of food origin and authenticity, characterize nutrients or chemical contaminants affecting human health, and ultimately, extend our understanding of food factors at the molecular level.

Scope and approach: This review focuses on the predominant MSI ionization technologies and summarizes their application to studies involving food science, including the imaging of food metabolites, elements, naturally occurring toxic constituents, and exogenous contaminates. Technical considerations associated with sample preparation, MALDI matrix choice and application, data processing, analyte identification, and spatial resolution are discussed, as are the future outlooks for MSI in food science.

Key findings and conclusions: MSI offers unparalleled chemical specificity for multiplexed analysis of the spatial distribution of nutrients, elements, and contaminants in food; information that is difficult or impossible to acquire with traditional staining or label-based methodologies. The unique spatio-chemical insights acquired with MSI have proven essential for understanding metabolic origin and change, and for visualization of exogenous substances having relevance to food quality and safety.

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1. Introduction

Monitoring and improving the safety and quality of our food is a universal theme for food scientists across the globe. From an analytical perspective, a goal is to meet the emerging demands of the agricultural and consumer communities through the expansion of existing measurement technologies and development of new ones. Although an impressive collection of methods has already been established, there remains a need for innovation that addresses the challenges associated with metabolite profiling in conventional and transgenic foods, rapid and accurate identification of known and unknown chemical contaminants, and for understanding food factors associated with human health. Consumers have begun to shift their attention away from strictly organoleptic characteristics to a greater focus on health and nutritional attributes (Bongoni, Steenbekkers, Verkerk, van Boekel, & Dekker, 2013). Therefore, food is no longer considered exclusively in terms of energy and satiation, but also for its impact on health and disease.

A variety of analytical tools are available for the assessment of food quality, safety, and traceability in modern food science. Primarily due to their outstanding sensitivity, precision, specificity, and short analysis times, modern mass spectrometry (MS)-based

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techniques, such as liquid chromatography (LC)–MS, gas chromatography (GC)–MS, capillary electrophoresis (CE)–MS, matrix-assisted laser desorption/ionization (MALDI) MS, and a variety of ambient ionization–MS systems, have become indispensable (Herrero, Simo, Garcia-Canas, Ibanez, & Cifuentes, 2012; Ibáñez, García-Cañas, Valdés, & Simó, 2013). Although chemical coverage and selectivity are unrivaled, separation-based experiments such as GC/LC/CE–MS require sample homogenization, extraction, and other manipulations. Furthermore, spatial information, which is important for understanding the complex involvement of various compounds in food quality, safety, and nutrition, is lost. Mass spectrometry imaging (MSI) has evolved over the past two decades to become a popular tool for untargeted, label-free, spatio-chemical characterization of biological systems (McDonnell & Heeren, 2007; Rubakhin & Sweedler, 2010). As shown in Fig. 1, a sample is generally fast frozen and sectioned in thin slices with a cryostat, and each slice is fixed onto an appropriate target plate by thaw-mounting. A computer-controlled sample stage, combined with various MS probes, can then be used to desorb/ionize analytes spot-by-spot or line-by-line over a defined sample area. The ions from each spot are extracted into the mass spectrometer for analysis, and two- or even three-dimensional chemical images corresponding to the original histological features can be generated by plotting the intensities of individual mass-to-charge ratio (m/z) peaks against the x-y coordinates. In contrast to MSI, conventional imaging techniques, e.g., immunohistochemistry, usually require time-consuming labeling of target analytes and are therefore relegated to the analysis of a limited number of preselected molecular components. Immunohistochemical methods are also problematic when imaging small molecules as these analytes are more prone to redistribution and cross-reactivity with antibodies.

MSI has been extensively employed to study normal and pathological animal tissues, to localize *in vivo* drug distributions in animal models, and to visualize the spatial distribution of plant secondary metabolites (Bjarnholt, Li, D'Alvise, & Janfelt, 2014; McDonnell & Heeren, 2007; Spengler, 2014; Stoeckli, Chaurand, Hallahan, & Caprioli, 2001); however, the use of MSI in food science is relatively recent (Handberg, Chingin, Wang, Dai, & Chen, 2014; Taira, Uematsu, Kaneko, & Katano, 2014), with approximately 50 manuscripts published to date. This imaging approach holds great potential for food science, allowing researchers to localize biomarkers of the origin and authenticity of food, and to characterize the endogenous metabolites or exogenous chemical compounds affecting human health at the molecular level. Here we highlight the predominant MSI approaches and techniques, review their application to important measurement issues related to food science and nutrition, and consider their future potential.

2. MSI techniques

Owing to the unique spatio-chemical information provided by MSI, a number of techniques, primarily differing in their approach to analyte desorption/ionization, have emerged to broaden the application and improve the performance of MSI in various research fields. The sample preparation and analyte desorption/ionization methods, mass analyzers, and other technologies used impact the results one obtains; thus, it is important to have a basic understanding of the available approaches when choosing the one most appropriate for an experiment. Consider also that many companies and universities will often have more than one MS system, and the recommendations provided by the staff will depend not only on the samples and data required by a user, but also on instrument availability. In the following sections we describe the fundamentals of several ionization techniques (also listed in Table 1), in roughly the order of their potential application to food science.

2.1. MALDI and related approaches

As a soft ionization technique, MALDI, along with electrospray ionization (ESI), delivers remarkable performance in biomolecular analysis. MALDI utilizes organic, light-absorbing matrices prior to laser irradiation, with either an ultra-violet (UV) or infrared (IR) laser beam, to irradiate the sample surface for desorption and ionization of target molecules. In brief, analyte-doped matrix crystals absorb the incident laser energy, which causes desorption from the sample surface into the gas phase, and where the combination of adiabatic expansion and gas-phase proton exchange reactions leads to ion formation (McDonnell & Heeren, 2007). The matrix is the first step of the MALDI imaging process, and as such, the choice of matrix and its successful application is directly correlated to analyte coverage, sensitivity, ionization efficiency, and the ultimate spatial resolution (see "*Choosing appropriate UV-MALDI matrices and deposition methods*" below). In contrast to UV-MALDI, the primary advantage of IR-MALDI is that endogenous water in the sample can act as a matrix to absorb the IR radiation.

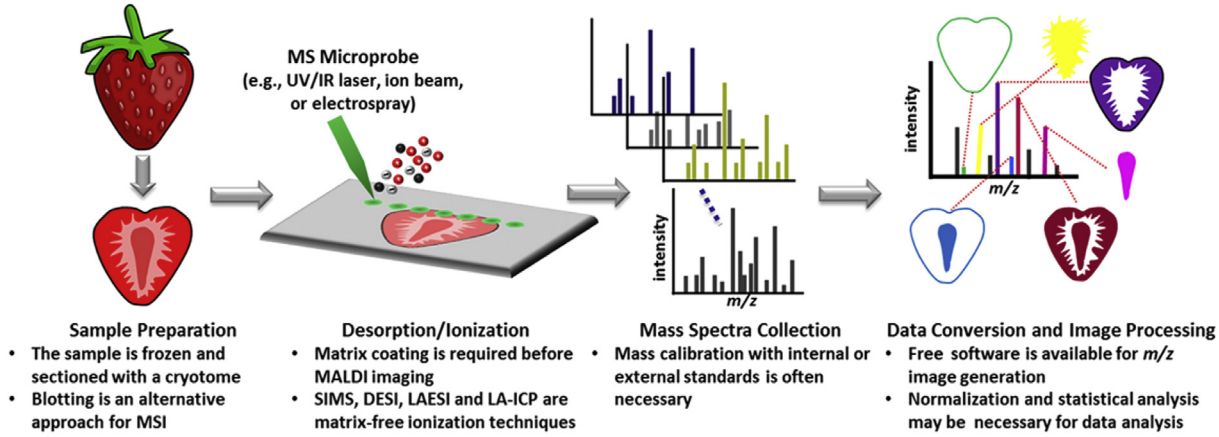

Fig. 1. Schematic illustration of a typical microprobe MSI experimental workflow. The frozen sample is cryosectioned and mounted on a suitable substrate. Following appropriate pretreatment (as dictated by the constraints of the ionization method and the sample at hand) the microprobe is sequentially rastered across the sample surface, generating a pixelated array of position-specific mass spectra. Using MSI software packages, individual ion abundances are plotted as a function of their location, generating a false color map that corresponds to the chemical distribution across the sample.

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