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MALDI mass spectrometry and imaging for lipid analysis in food matrices: A review of analytical principles and perspectives

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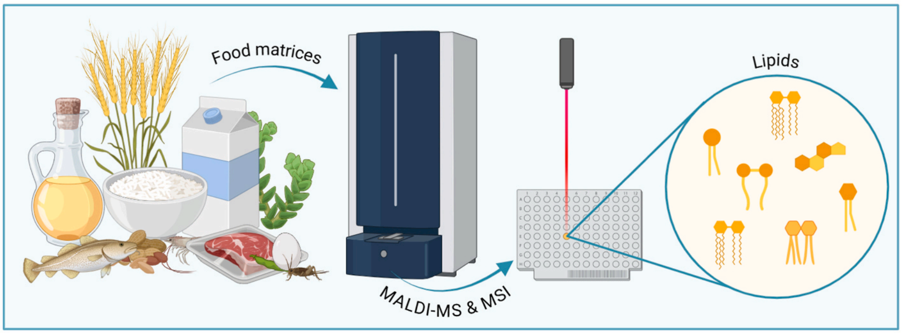
HIGHLIGHTS

- Lipidomics helps detect food fraud and understand food-related processes.
- MALDI-MS enables fast, direct lipid analysis with minimal preparation.
- Glycerolipids are the most studied lipid class in food-related MALDI studies.
- 2,5-dihydroxybenzoic acid is the most used MALDI matrix for lipid analysis in food.
- MALDI-MSI enables in situ lipid mapping without complex sample preparation.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Lipids play essential roles in food systems, and their study through lipidomics offers insights into biochemical processes and issues such as food fraud and adulteration. Among lipid classes, glycerolipids (GLs), particularly triacylglycerols (TAGs), are the most frequently analysed in food-related studies.

Results: Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and MALDI mass spectrometry imaging (MSI) have emerged as powerful tools for lipidomic investigations. While traditionally applied in biological research, their use in food science is expanding to diverse matrices, including oils, dairy, cereals, algae, and meat. In terms of MALDI matrices, 2,5-dihydroxybenzoic acid (DHB) is most commonly used in positive ion mode, whereas 9-aminoacridine (9-AA) is typically employed in negative mode. MALDI-MS has advanced lipid characterization, and MALDI-MSI now enables mapping of lipid spatial distributions, revealing processes such as lipid degradation.

Significance: This review underscores the growing utility of MALDI-MS and MALDI-MSI for lipid analysis in food science. We highlight their analytical capabilities, workflows, and limitations, it demonstrates how these techniques can enhance understanding of food composition, quality, and authenticity.

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1. Introduction

Lipids play a central role in nutrition and physiology, serving as a primary source of energy through the oxidation of fatty acids. They also function as highly efficient energy reserves, particularly in the form of stored fat, which contains minimal water compared to other macronutrients like proteins or carbohydrates. Beyond their energetic value, lipids are essential components of biological systems, as structural elements of cellular membranes, signalling messengers or disease biomarkers [1]. The term “lipidome” was introduced in 2001 [2], referring to the complete set of lipids in a biological sample and the quantitative comparison of lipid species between different physiological or pathological states. From this term derives “lipidomics”, which is generally defined, following Franz Spener [3], as the comprehensive characterization of lipid molecular species and their biological roles, including the involvement of proteins in lipid metabolism and regulation at the genetic level. This belongs to a group formed by several approaches known as “omics” which have gained substantial relevance due to their ability to generate comprehensive datasets. These include fields such as proteomics, genomics and, more recently, interactomics [4]. They are strongly related with the traceability of specific molecular markers which is crucial for monitoring both identity and quality traits. Besides biological information about the systems studied and their temporal modifications, these marker biomolecules can be used for both authentication purposes and quantifying adulteration levels, based on their signals and their intensities [5].

Food fraud is a multifaceted issue that continues to pose significant challenges across the global food supply chain. It includes a wide range of deceptive practices carried out with the intent of economic gain, often at the expense of consumer trust and safety [6]. Among the affected categories are oils, dairy products, fish, meat, honey, alcoholic beverages, fruit juices, spices, and grain-based foods, many of which are high-value or subject to complex international trade [7]. Adulteration remains the most common form of fraud and can take several forms: substitution of valuable ingredients with cheaper alternatives, dilution of a product with lower-quality material, unapproved enhancement with undeclared additives to mask inferior quality, or concealment of the quality of the ingredients [7]. Other forms include mislabelling, false claims on packaging such as origin, organic certification, or nutritional value, counterfeiting of branded goods, and unauthorized distribution through grey market channels. These practices not only undermine fair market practices but can also endanger consumer health when harmful or allergenic substances are involved.

The evolving nature of fraud, driven by increasingly sophisticated methods of deception, highlights the urgent need for continuous monitoring and enforcement. As detection methods improve, fraudulent actors also adapt, making prevention and control a moving target [5]. Spectroscopic techniques, including nuclear magnetic resonance, near- and mid-infrared, Raman, ultraviolet–visible, and X-ray fluorescence spectroscopy, have been widely employed for successful non-targeted analytical approaches directed to detecting food fraud [7]. As an example, extra virgin olive oils have been analysed by Fourier-transform infrared spectroscopy, successfully demonstrating if they had been adulterated with other low-cost edible oils. However, mass spectrometry (MS) has emerged as the preferred technique in lipidomics research due to its remarkable sensitivity, specificity, and ability to detect, identify, and quantify lipids even in complex matrices [8]. Advances in MS instrumentation have led to the development of a extensive range of methodologies for assessing food quality, authenticity, and safety. Indeed, most omics-based approaches rely heavily on MS technologies, which have become foundational tools across disciplines such as physics, chemistry, biochemistry, and increasingly, clinical diagnostics [4]. In this context, different techniques such as electrospray ionization (ESI), ambient mass spectrometry (AMS), and high-resolution mass spectrometry (HRMS) have been already applied for non-targeted analyses [7,9,10]. Among these techniques, matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) and matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) stand out as a particularly attractive option for rapid and accurate screening in food fraud detection, owing to its user-friendly operation, speed, and capacity to handle complex matrices with minimal preparation [7]. MALDI-based techniques have already proven their usefulness in food authentication by enabling lipid fingerprinting approaches. For instance, England et al. [11] demonstrated that MALDI-TOF MS could discriminate bovine milk from non-dairy alternatives based on differences in their lipid profiles, without the need for extensive sample preparation, thus providing a rapid tool to detect adulteration. Similarly, in the case of olive oils, Alves et al. [12] applied this technique to profile triacylglycerols (TAGs). The resulting spectra provided characteristic molecular signatures that allowed not only the differentiation of extra virgin olive oils from virgin olive oil varieties. Another example is the finding that freeze-drying can preserve human milk stability over time, which means it could be used in emergency situations without danger [13].

This review focuses on the application of MALDI-MS and MALDI-MSI as analytical tools for the identification of lipids in food matrices. Both techniques are explored in terms of their working principles, analytical capabilities, and their specific utility in food analysis. The discussion is structured around two main sections, corresponding to each technique, and further organized according to sample preparation and detection. While MALDI-MS is not generally considered the most comprehensive method for lipid analysis when imaging is not involved, this review aims to demonstrate how the technique can still be effectively leveraged for lipidomic applications, while also critically addressing its limitations. Importantly, to the best of our knowledge, no up-to-date reviews have been published that specifically address lipid analysis in food systems, nor do existing works adequately cover their potential in the context of food adulteration. Furthermore, reviews of MALDI applications that are available are either broad in scope or focused on some other specific aspects such as MALDI matrix selection and development, certain lipidic families or other biomolecules such as proteins, often lacking a complete picture of the analytical workflow [14–20]. In this sense, the present review provides a novel and timely perspective by combining lipidomics, food analysis, and fraud detection within the framework of MALDI-based techniques.

2. Methodology of the literature review

This review examines recent advances in the application of MALDI-MS and MALDI-MSI for lipid analysis in food matrices. Relevant literature was searched in Scopus, Web of Science, ScienceDirect, and Google Scholar using the following specific keyword combination: “MALDI-TOF” OR “Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight” OR “MALDI-MSI” OR “MALDI Imaging” AND “lipid” AND “food”. The selection was limited to studies published in English between 2000 and 2025. Full-text articles meeting the inclusion criteria were retrieved, and their reference lists were manually screened to uncover additional pertinent studies that may not have been identified during the initial database search.

MALDI-based techniques have not yet been widely applied to food matrices (38 articles since 2000), particularly in the case of imaging applications. However, the reviewed studies clearly demonstrate the high potential and usefulness of these techniques in food analysis. A broad range of food matrices have been investigated, from liquid samples such as oils [21–23] and milk [11,13,24–28] to more complex solid matrices including fish [29,30] and bull meat [31]. The choice of MALDI matrices for lipid analysis on food samples has also been diverse (see Fig. 1A), nonetheless, three matrices, namely 2,5-dihydroxybenzoic acid (DHB) (53 %), 9-aminoacridine (9-AA) (18 %), and α-cyano-4-hydroxycinnamic acid (CHCA) (13 %) predominate over 1,5-diaminonaphthalene (DAN) (5 %). Regarding the lipid classes detected in the reviewed articles, all major families, according to the LIPID Metabolites and

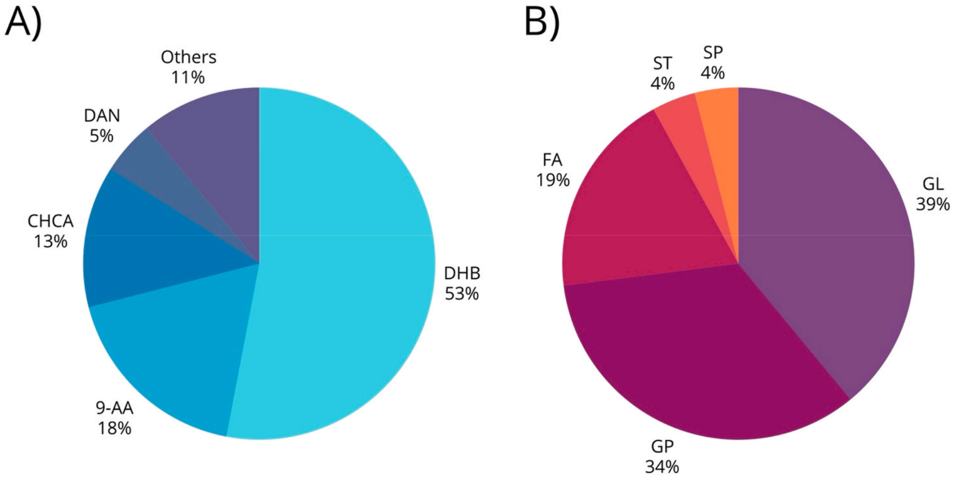

Fig. 1. Overview of (A) the most commonly used MALDI matrices and (B) lipid families detected in the reviewed literature, based on the LIPID MAPS® classification [22]. 9-AA, 9-aminoacridine; CHCA, α-cyano-4-hydroxycinnamic acid; DAN, 1,5-diaminonaphthalene; DHB, 2,5-dihydroxybenzoic acid; FA, fatty acyls; GL, glycerolipids; GP, glycerophospholipids; SP, sphingolipids; ST, sterol lipids.

Pathway Strategy (LIPID MAPS®) classification [32], have been analysed (see Fig. 1B), with glycerolipids (GLs) (39 %) and glycerophospholipids (GPs) (34 %) being the most frequently studied, as will be further detailed in Section 3.

3. Lipid classes & presence in food matrices

Lipids are broadly defined as a diverse group of hydrophobic or amphipathic molecules that are generally insoluble in water but dissolve readily in nonpolar solvents such as chloroform, hydrocarbons, and alcohols. However, this general definition does not cover all structurally related compounds. Unlike other macronutrients such as proteins and carbohydrates, lipids lack a unifying structural motif, which complicates their classification. They can be categorized based on various criteria, including their physical state at ambient temperature, polarity, biological essentiality, or chemical complexity [33]. LIPID MAPS® [32] provides a widely accepted classification framework, dividing lipids into eight major categories including fatty acyls (FA), glycerolipids (GL), glycerophospholipids (GP), sphingolipids (SP), sterol lipids (ST), prenol lipids (PR), saccharolipids (SL), and polyketides (PK). Each group includes subclasses that differ in structural features such as polarity, charge, functional groups, and backbone linkages, as can be seen in Table 1. As of July 2025, the LIPID MAPS® Structure Database (LMSD) [32] catalogs over 49,790 unique lipid structures.

Lipids that can be obtained from foods primarily consist of oils and fats, the majority of which are TAGs, in the GL family, which can account for up to 99 % of the total lipid content in both plant- and animal-derived products. GPs, while quantitatively minor in some oils due to their removal during refining processes, remain significant structural lipids in other unrefined food matrices. For instance, in foods like eggs, meat, and fish, phospholipids represent a considerable proportion of total lipids [34]. In addition to these, edible oils can contain smaller quantities of other lipid components, including cholesterol, cholesterol esters (CE), and fat-soluble micronutrients such as vitamins and polyphenols [33].

As shown in Tables 2 and 3 and Fig. 1B, our findings are consistent with the previously discussed trends. GLs, are the most frequently analysed lipid class in food-related studies, representing nearly 40 % of the reviewed literature, and they include TAGs in most of their studies. TAGs are detected across a broad spectrum of food matrices, including

Table 1
Main lipid families and their subclasses according to LIPID MAPS® database[a] [66].

Fatty Acyls [FA]	Glycerophospholipids [GP]	Sterol Lipids [ST]	Sphingolipids [SP]	Glycerolipids [GL]
Fatty acids/esters (FA)	Glycerophosphocholines (PC)	Sterols, inc. bile acids (ST)	Sphingoid bases (SPB)	Tri (acyl|alkyl)glycerols (TAG) (TG)
Acyl carnitines (CAR)	Glycerophosphates (PA)	Cholesterol esters (CE)	Sphingoid base phosphates (SPBP)	Di (acyl|alkyl)glycerols (DG)
Acyl CoAs (CoA)	Glycerophosphoserines (PS)		Ceramides (Cer)	Mono(acyl|alkyl)glycerols (MG)
N-acyl ethanolamines (NAE)	Glycerophosphoethanolamines (PE)		1-O-acyl ceramides (ACer)	Monogalactosyldiacylglycerols (MGDG)
N-acyl taurines (NAT)	Glycerophosphoglycerols (PG)		Ceramide phosphates (CerP)	Digalactosyldiacylglycerols (DGDG)
	Glycerophosphoinositols (PI)		PI-ceramides (IPC)	Sulfoquinovosyldiacylglycerols (SQDG)
	Glycerophosphoinositol phosphates (PIP)		PE-ceramides (CerPE)	Diacylglyceryl-hydroxy-methyltrimethylalanine (DGTA)
	Cardiolipins (CL)		Sphingomyelins (SM)	Diacylglyceryltrimethylhomoserine (DGTS)
	Bis(monoacylglycerol)phosphates (BMP)		Hexosyl ceramides (HexCer)	Diacylglycerolcarboxyhydroxymethylcholine (DGCC)
	Glycerophosphoethanols (PEth)		Mannosyl-PI-ceramides (MIPC)	
	Glycerophosphothreonines (PT)		Mannosyl-di-PI-ceramides (M(IP)2C)	
			Dihexosyl ceramides (Hex2Cer)	
			Sulfatides (SHexCer)	
			Glycosphingolipids (Various)	

[a] Not Including Polyketides [PK], Prenol Lipids [PR] and Saccharolipids [SL], as LIPID MAPS® Does Not Provide Their Subclasses.

Table 2
Overview of research articles using MALDI-MS for lipid analysis in food products.

Food Sample	Lipid Family[a]	MALDI Matrix	Extraction Solvent	Sample Amount	Ion Mode	Range	Ref.
Oil	GL	DHB	Pentane	50 mg	+/−	500–1100	[39]
Bee Pollen	NS	CHCA	AA in H_2O, ACN and TFA in H_2O	12 mg	+	600–18000	[51]
Fish	FA	NS	$CHCl_3$:MeOH (2:1)	230–300 mg	+/−	NS	[29]
Oil, Pâté	GL	DHB	THF	3 g	+	240–2400	[50]
Milk	GP	DHB,	$CHCl_3$:MeOH (2:1)	2 ml, 2 mg	+	200–1600	[25]
Oils	FA	F20TPP	MeOH:H_2O (4:1)	20 mg	−	NS	[43]
Algae	FA, GP, GL	DAN, 9-AA	$CHCl_3$:MeOH (2:1)	200 mg	+	NS	[35]
Fish	GP	SA, DHB	NS	NS	+	700–100	[30]
Milk	GP, GL	sDHB	H_2O	1 ml	+	400–1000	[11]
Milk	FA, GP	CHCA, DHB	MeOH, MTBE	20 μL	+	400–1000	[24]
Milk	NS	DHB, SA	NS	1 μL	NS	600–5000	[13]
Milk	FA	DHB	diisopropylether:1-butanol (6:4)	10 mL	NS	NS	[26]
Cricket	GL, ST, FA	NS	DCM:MeOH (2:1)	5 g	+	NS	[36]
Oil	FA	DHB	$CHCl_3$	5 mg	NS	NS	[67]
Animal Fat	GL	DHB	$CHCl_3$	10 mg	+/−	NS	[42]
Egg Yolk	GP	DHB, 9-AA	$CHCl_3$:MeOH:H_2O (1:1:1)	NS	+/−	NS	[40]
Oils	GL	DHB	$CHCl_3$	10 mg	NS	NS	[12]
Algae	GL	sDHB	NS	NS	+	NS	[65]
Oil	GL	DHB, CHCA	No	NS	+	500–2000	[23]
Oils	GL	DHB	HX	1 mg	+	NS	[49]
Oils	GL	THAP	$CHCl_3$	NS	+/−	800–4000	[22]
Milk	FA, GP, SP	CClCA, CHCA	$CHCl_3$:MeOH (1:2), H_2O: $CHCl_3$(1:1)	50 μL	NS	500–2000	[27]
Milk	FA	DHB	$CHCl_3$	10 mL	+	400–1500	[28]
Egg Yolk	GP, SP, GL	Di-FCCA, 9-AA	$CHCl_3$:MeOH:H_2O (1:1:1)	NS	+/−	NS	[68]
Bull Muscle	GL	DHB, 9-AA	$CHCl_3$:MeOH (2:1)	2 g	−	NS	[31]
Algae	GL	DHB	$CHCl_3$:MeOH (2:1)	NS	+	NS	[41]
White Shrimp	GP	DHB	H_2O with salts	NS	+	500–1000	[69]
Oils	GL	DHB	$CHCl_3$	1 mg	+	NS	[21]

[a] Classification according to LIPID MAPS® Database. **9-AA**, 9-aminoacridine; **CClCA**, 4-chloro-α-cyano-cinnamic acid; **CHCA**, α-cyano-4-hydroxycinnamic acid; **$CHCl_3$**, chloroform; **DAN**, 1,5-diaminonaphthalene; **DCM**, dichloromethane; **DHAP**, 3′,5′-dimethoxy-4-hydroxyacetophenone; **DHB**, 2,5-dihydroxybenzoic acid; **Di-FCCA**, difluorocinnamic acid; **FA**, fatty acyls; **F20TPP**, *meso*-tetrakis (pentafluorophenyl)porphyrin; **GL**, glycerolipids; **GP**, glycerophospholipids; **H_2O**, water; **HX**, hexane; **IPA**, isopropanol; **MeOH**, methanol; **MTBE**, methyl *tert*-butyl ether; **NS**, not specified; **sDHB**, super-DHB; **SA**, sinapinic acid; **SP**, sphingolipids; **ST**, sterol lipids; **TFA**, trichloroacetic acid; **THAP**, 2′,4′,6′-trihydroxyacetophenone; **THF**, tetrahydrofuran.

oils, algae, milk, and cereals, and are often the sole lipid species examined within this category. Among these, olive oil and sunflower seed oil blends have been studied in terms of their TAG composition, as they are some of the most common blended products in the market nowadays. Fig. 2 displays the MALDI-MS spectra of pure olive oil, pure sunflower seed oil, and their blends at different mixing ratios. Noticeable variations in peak intensities within the TAG region reflect the changing oil compositions, which demonstrates the correlation between the blending ratio and the lipid composition revealed by MALDI-MS analysis. In the spectrum of pure sunflower seed oil, the dominant ions were detected at m/z 901.7 (LLL, L = linoleic acid) and m/z 903.7 (OLL, O = oleic acid), while olive oil exhibited higher signals at m/z 881.8 (POO, P = palmitic acid) and m/z 907.8 (OOO). As the proportion of olive oil increased in the blends, the relative intensities of m/z 881.8 and m/z 907.8 rose, whereas those at m/z 901.7 and m/z 903.7 diminished [23].

Other members of the GL family, such as diacylglycerols (DG), digalactosyldiacylglycerols (DGDGs), and sulfoquinovosyldiacylglycerols (SQDGs), are also analysed but with less frequency [35]. GPs are the second most studied group, accounting for approximately 34 % of the articles. Similar to GLs, they are assessed across a diverse range of food products and are frequently studied alongside these to provide a more comprehensive profile of glycerol-based lipids. FAs rank third, comprising 19 % of the analyses, and are predominantly investigated in high-fat food matrices such as oils, milk, and meat. This group also includes polyunsaturated fatty acids (PUFAs), which are of particular interest due to their roles as antioxidants and anti-inflammatory lipid mediators [35]. In contrast, STs and SPs are much less commonly studied, each accounting for only 4 % of the reviewed studies.

It is important to note that not all lipid analyses are aimed at biomarker discovery. In many cases, studies focus on known lipid compounds to assess their presence or monitor changes in lipid composition following specific treatments or processes. Consequently, a comprehensive lipidomic profile is not always carried out. STs and SPs are typically analysed with the more abundant lipid families, rather than as standalone targets. This approach likely reflects the need for more in-depth characterization when these minor lipid classes are of interest. For example, STs are frequently investigated due to their association with cholesterol and are usually motivated by its physiological implications. For example, as noted by Tzompa-Sosa et al. [36], phytosterols in particular are considered functional ingredients due to their potential to improve the lipoprotein profile by lowering LDL cholesterol levels and reducing intestinal cholesterol absorption. Regarding SPs, sphingomyelin is the main compound of interest in the reviewed studies. As a major sphingolipid component of mammalian cell membranes, its presence in animal-derived products such as milk and eggs supports its selection as a target analyte in these matrices. In the context of MALDI-MSI, it should be stated that the same lipids cannot be indiscriminately selected across different regions of a given matrix. Studies focusing on the spatial distribution of analytes often aim to evaluate either distribution or heterogeneity after processes such as degradation [37]. This will be further explained in Section 5.

4. MALDI mass spectrometry in food

4.1. Fundamentals of MALDI-MS

MALDI is a soft ionization technique in MS [21,31,37–41], widely employed since the 1980s for the analysis of biomolecules. Its early success stemmed from its ability to ionize large, fragile molecules such as proteins without inducing significant fragmentation. Over time, its applicability has expanded to include a large variety of analytes, including peptides, glycans, oligonucleotides, lipids, metabolites, and synthetic polymers [5].

The core principle of MALDI (see Fig. 3) relies on the use of a matrix capable of absorbing most of the energy of the laser, which is usually in UV or IR wavelengths, depending on the material on which the laser is

Table 3
Overview of research articles using MALDI-MSI for lipid analysis in food products.

Food Sample	Lipid Family[a]	MALDI Matrix	Extraction Solvent	Sample Amount	Ion Mode	Embedding Medium	Thickness (μm)	Matrix Deposition	Laser Intensity (%)	Range	Laser Type	Frequency (Hz)	Ref.
Rice, Wheat	GL, GP	9-AA, DHB	MeOH	0.2 g	NS	10 % gelatin	20	Sublimation	16–25	NS	Nd:YAG	1000	[38]
Oat	GL, GP	CHCA	MTBE:MeOH (3:1) or $CHCl_3$:MeOH (2:1)	50 mg	+/−	10 % gelatin	25	Spraying	NS	300–1300	NS	NS	[70]
Beans	GP	DHB	MTBE:MeOH (1:1)	5 mg	NS	NS	15	Sublimation	NS	400–2000	NS	200	[63]
Meat	GP, FA, GL, ST	CHCA,9-AA	$CHCl_3$:MeOH (2:1)	10 g	+	NS	16	Spraying	NS	300–1660	NS	200	[37]
Rice	GP	DHB	No	NS	+/−	10 % gelatin	16	Sublimation	25	500–1000	Nd:YAG	1000	[44]
Lentil	GP, GL	CHCA, 9-AA	No	NS	+	10 % gelatin	45	Sublimation	10–50	500–1000	Nd:YAG	1000	[45]
Fish	GP	DAN	No	NS	−	NS	10	Spraying	50	400–900	NS	NS	[60]
Peanut Seeds	GL, GP	DHB	No	NS	+	10 % gelatin	8	Spraying	80	50–1300	NS	NS	[62]

[a] Classification according to LIPID MAPS® Database. **9-AA**, 9-aminoacridine; **CHCA**, α-cyano-4-hydroxycinnamic acid; **DAN**, 1,5-diaminonaphthalene; **DHB**, 2,5-dihydroxybenzoic acid; **FA**, fatty acyls; **GL**, glycerolipids; **GP**, glycerophospholipids; **MeOH**, methanol; **MTBE**, methyl *tert*-butyl ether; **NS**, not specified; **ST**, sterol lipids.

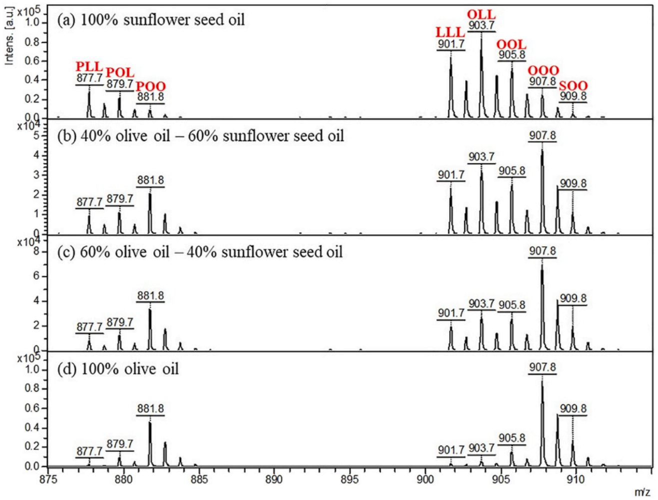

Fig. 2. TAG region of the MALDI-MS spectra for (a) 100 % sunflower seed oil, (b) 40 % olive oil - 60 % sunflower seed oil blend, (c) 60 % olive oil - 40 % sunflower seed oil blend, and (d) 100 % olive oil, with the main peaks assigned. P, palmitic acid; L, linoleic acid; O, oleic acid; S, stearic acid. Reprinted from Ref. [23], with permission from Elsevier.

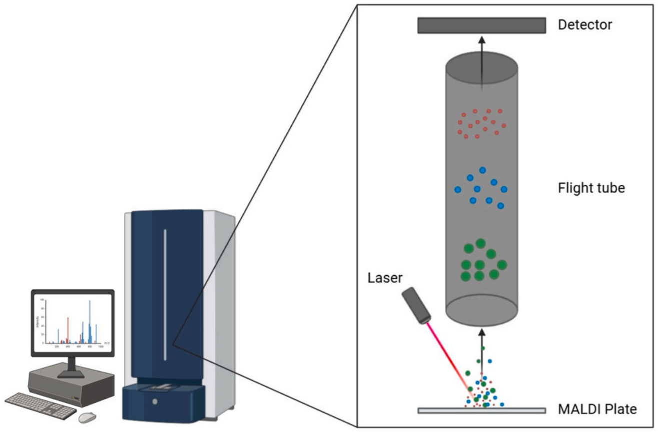

Fig. 3. Schematic representation of the MALDI-TOF MS working principle.

based on. Most used lasers in food analysis in the last years have been N_2 [12,39,42,43], Nd:YAG [22,38,44,45] and Nd:YLF [35]. MALDI allows for the formation of positive and negative ions, although the latter are analysed considerably less when determining lipids in food matrices as can be seen in Table 2, accounting for only 8 % of the reviewed articles on its own. This is likely due to the lower detectability of lipids in this ionization mode. The ionizing agents used in this mode will be discussed in Section 4.3. Because MALDI uses a pulsed laser sequence, the technique is commonly coupled with time-of-flight (TOF) mass analysers, and so it is usually referred to as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) [46]. Key advantages of MALDI-TOF MS are that it offers a simple, rapid, and straightforward approach, requiring minimal sample preparation steps. It is highly sensitive, and robust, with the versatility to analyze a broad range of compounds in terms of both molecular weight and polarity. It also presents high tolerance to impurities, high throughput capability, ease of automation, and cost-effectiveness. In terms of reproducibility, this technique has the potential to achieve high consistency, as analyses are typically based on the average of multiple laser shots. However, proper mixing of the sample and matrix, as well as accurate matrix deposition (to minimize crystal variability), are essential. This aspect is also closely related to quantitation, where the technique still faces well-known challenges. The inherently non-quantitative nature of MALDI-TOF MS has been widely discussed in the literature, as variability in signal intensity caused by so-called “hot spots” or “sweet spots” leads to poor reproducibility between measurements. Such inconsistencies are typically linked to variations in the quantity and crystallization behavior of the matrix material, which can differ substantially between formulations. For instance, matrices like CHCA tend to produce relatively uniform crystal layers and therefore reduce these effects [47]. Other of the most common strategies to mitigate poor reproducibility involves the use of internal standards, with isotopically labelled analogs of the analyte [48].

Finally, it should be commented that the implementation of MALDI can generally be divided into two main stages. The first involves initial sample preparation, which may or may not require a prior extraction step, depending on the nature of the sample and the target analytes. This step aims to obtain a suitable solution or material form that can be effectively analysed. The second stage concerns the selection and optimization of detection parameters, including all steps following sample deposition onto the MALDI target plate. This includes matrix selection and application, ionization conditions, laser settings, and data acquisition parameters, all of which are critical to achieving accurate and reproducible results.

4.2. Sample preparation techniques

Due to the distinct chemical properties of lipid molecules, these aqueous systems used for protein extraction are not often applicable to lipidomics. Lipid analysis typically requires non-polar extraction solvents and specialized ionization strategies to accommodate the hydrophobic nature of lipids. Additionally, lipidomics must account for the potential aggregation of lipid species under specific conditions, a characteristic that further differentiates it from other omics approaches [14]. This all leads to considerable differences between sample preparation methods. The term “food” refers to a broad variety of analytical matrices that differ significantly from one another. As a result, the procedures required to extract analytes also vary depending on the specific matrix.

For relatively simple matrices with high lipid content, such as edible oils, direct dissolution in nonpolar solvents is often sufficient. For instance, vegetable oils have been successfully dissolved in solvents like pentane with vortex agitation for 1 min [39] or in hexane [49]. Similarly, milk can be solubilized using methanol [26], which facilitates a straightforward extraction process. In other instances, even less laborious methods have been used; for example, diluted matrix solutions have been directly deposited onto the well together with the sample. In the case of milk, this was done using an aqueous solution [11], while for oil samples the authors used tetrahydrofuran [50] or chloroform [21]. An even more minimal approach has been reported where a thin film of oil was spread directly onto a pre-deposited DHB matrix layer using a cotton tip [23]. Nevertheless, the choice of extraction strategy strongly depends on the target lipid class. For example, when the goal is to analyze free fatty acids specifically, alternative chemical treatments such as saponification have been applied in the previous matrices [28,43].

More complex or heterogeneous matrices, such as edible crickets, often require more elaborate protocols like Folch extraction [36]. This method involves the use of a solvent mixture to form biphasic systems, typically requiring extended vortexing (20 min) followed by a long resting period (up to 12 h) to allow phase separation. Furthermore, in this case it used relatively large amounts of sample (e.g., 5 g) and involves toxic solvents like dichloromethane, employed for additional cleaning steps. Nonetheless, this protocol has also been adapted for milk by Walczak et al., with relatively reduced sample amounts (2 g or 2 mL for powder and liquid milk, respectively), and followed by solid-phase extraction (SPE) for further refinement [25]. Other food matrices, such as bee pollen, present additional challenges due to their dense and resistant structures. In these cases, mild lysis-like procedures may be necessary. Formic acid treatment has been used to break down pollen balls and extract lipid content more effectively [51]. This is also reflected in the case of meat-based matrices. For instance, Dannenberger et al. [31] performed lipid extraction from 2 g of muscle tissue using 15 mL of a chloroform:methanol mixture (2:1, v/v), following the Folch method. The extraction was carried out by homogenizing the sample with an Ultra-Turrax (three cycles of 15 s at 12,000 rpm) at room temperature. After solvent evaporation, the extracted material was quantified by gravimetric analysis. The lipids were then re-dissolved in the same mixture.

These observations underscore the importance of the food matrix in determining the most suitable sample preparation approach. Despite these variations in the number and type of preparatory steps, the goal remains the same: to isolate a lipid-rich extract suitable for subsequent MALDI analysis. Ultimately, all methodologies converge on obtaining a final lipidic fraction that contains the target analytes in a form compatible with the subsequent ionization and detection.

4.3. Detection for MALDI lipid analysis in food

Detection in MALDI-MS is a critical step that directly impacts the quality and reliability of the analysis. Several factors influence detection efficiency, including the choice of matrix, the addition of ionizing agents, and their ratio with the sample. The matrix must not only facilitate ionization but also be compatible with the specific lipid class and sample complexity. Similarly, the type and concentration of ionizing agents can enhance or hinder signal intensity. Moreover, relative proportion between matrix, ionizing agents, and analyte must be optimized, as imbalances can lead to poor ionization efficiency or suppression effects [5].

In terms of the MALDI matrix, efforts have been made in order to predict suitable ones theoretically, but most have historically been discovered through trial and error or serendipity. A key requirement for a matrix is strong absorption at the laser wavelength, which is typically around 337 or 355 nm for UV lasers so it can efficiently absorb laser energy and facilitate analyte ionization. In addition, an ideal matrix should crystallize homogeneously to ensure consistent ionization and signal reproducibility. It must also remain stable under the high vacuum conditions of the mass spectrometer, as instability or sublimation can lead to time-dependent variations in signal quality and analyte detection. Additionally, it should provide a high sensitivity with a strong signal-to-noise ratio for the analytes of interest. However, matrix oligomers frequently produce characteristic peaks at higher mass-to-charge ratios than the original matrix, which can sometimes serve as internal calibration markers. Finally, MALDI matrix should produce primarily a single quasi-molecular ion of the analyte which helps simplify data analysis and interpretation [4,18,20]. The choice of matrix depends strongly on which kind of lipids the analysis intends to determine. Carboxylic acids—particularly derivatives of cinnamic and benzoic acids—are commonly used as MALDI matrices because of their acidic nature which promotes the formation of $[M+H]^+$ ions. Sodium adducts are also frequently observed, given sodium’s widespread presence as a contaminant. Among the dihydroxybenzoic acid isomers, DHB is especially favored due to its strong UV absorption and efficient ionization performance compared to other isomers. As can be seen in Table 2 and Fig. 1B, DHB and super-DHB (9:1 mixture of DHB and 2-hydroxy-5-methoxybenzoic acid, w/w) are in fact the most applied matrices for lipid analysis in food matrices as well, accounting for 53 % of the reviewed literature. DHB has been widely employed, for instance, in the analysis of glycerolipids, where it provides good ionization efficiency and reproducibility. However, its suitability can vary significantly depending on the lipid class under study. For example, its lithium salt (LiDHB) has been reported to be a more appropriate matrix for the analysis of hydrocarbons than the free acid form, as it enhances cationization and improves spectral quality. In contrast, for free fatty acids, this matrix did not generally yield satisfactory results, as these compounds tend to ionize poorly under standard conditions. Similarly, cholesterol is typically not efficiently detected when using DHB as the matrix [4]. 9-AA on the other hand is mostly used for negative ionization since it was proposed for its better sensitivity and reduction of suppression effects compared to other matrices [52]. This has proven to be effective for the analysis of phospholipids and TAGs and has shown good performance in the detection of free fatty acids [4]. Other matrices such as CHCA or DAN have also been reported in some of the reviewed articles. As for the ratio, it is mostly not specified in articles, but it is well established that it should be at least 1:1 (sample:matrix, v/v) or with a greater matrix proportion [4].

Besides the simple protonation processes the sample might go through on its own, ionizing agents can assist in the formation of quasi-molecular ions by attaching to the analyte molecules, thereby improving detection sensitivity and signal clarity. These agents might typically be either cations, such as sodium (Na^+) or silver (Ag^+), or anions, like iodide (I^-). Sometimes, ionizing agents are added directly as salts to the sample or matrix mixture [1,53,54], for example, silver trifluoroacetate (AgTFA). This salt has been used with DHB as the matrix for determining squalene, as it is an apolar compound that is difficult to determine in positive mode using other conditions [4]. Most commonly, salts come from trifluoroacetic acid (TFA) because not only is this a comparatively weakly coordinating ion, which facilitates analyte dissociation, but it also possesses several other advantageous properties. TFA-derived adducts, such as $[M + TFA + 2\,Na]^+$, are easier to identify since TFA is rarely present as a contaminant in typical samples. Additionally, fluorinated compounds like TFA tend to exhibit greater volatility than their non-fluorinated analogs, aiding in desorption and ionization [55]. TFA is also UV-transparent at the most commonly used MALDI excitation wavelengths, reducing background interference [56]. Furthermore, TFA salts are less hygroscopic than other weakly coordinating counterions, making them more stable and easier to store. Be that as it may, in certain instances, the ionizing agent is introduced as part of the matrix compound itself, like Li^+ in lithium salts of DHB. Ionizing agents are rarely seen in the reviewed articles, which might be caused by the fact that the ionization is frequently enough on its own.

5. MALDI mass spectrometry imaging in food

5.1. *Fundamentals of MALDI-MSI*

Most of the concepts discussed in Section 4.1 regarding the basis of MALDI-MS also apply here, as the core technique remains largely the same. Therefore, those details will not be reiterated in this section. Therefore, the main difference between both techniques lies in the fact that in MALDI-MSI the laser is not directed at a specific spot in the sample, but rather it scans across it, generating ions that are then identified and mapped based on their mass-to-charge ratios, and then transform the information into an image in which pixels are colored according to said ions [57–59]. In Fig. 4, two images from 2014 [37] and 2023 [38] are presented illustrating the contrast in quality. In Fig. 4A, a section of steak is shown where the distribution of lysophosphatidylcholine was monitored at different storage times using a spatial resolution of 200 μm. On the other hand, in Fig. 4B, a rice grain section is presented, displaying several lipid species (indicated in the image by their *m/z* values) under different sample preparation conditions, with a spatial resolution of 35 × 35 μm. The resolution has been significantly improved in these years [60], as it can be appreciated only by looking at the images, although this spatial resolution in MALDI is often constrained by the diameter of the laser beam and the properties of the matrix crystals can also play a role, leading to fluctuations in ionization efficiency [61].

It should be remarked that the main advantage of MALDI-MSI against “traditional” MALDI-MS is that the latter is applied to homogenized extracts, which, as expected, results in the loss of crucial information about the original spatial distribution of lipids within the sample [37,45,60,62]. If the matrix under study is, for example, oil [21–23] or milk [11,13,24–28], this is not a significant issue, as the distribution of lipids in liquid matrices tends to be homogeneous. A similar rationale applies to analyses aimed at determining the overall lipid content or generating tentative lipid profiles, where spatial localization is not the primary focus. However, understanding where these compounds are located is in many cases essential for understanding their complex and vital functions in biological systems [8]. For example, rice [44], beans [63] or seeds [62], are matrices in which the internal lipid distribution may differ significantly from that on the surface. Another important and occasionally localized process is degradation, which when occurring in specific areas of food matrices plays a significant role in shaping consumer perception. For instance, a meat cut may be largely of good quality, while the outer layer shows signs of deterioration. Understanding how lipids degrade over time is essential, as it impacts not only storage and transport conditions but also product quality, preparation characteristics, functional properties, and ultimately, shelf life [37]. Workflows including MALDI-MSI consist mainly of three main steps: sample preparation (including tissue embedding and cryosectioning), matrix deposition and data acquisition and visualization (see Fig. 5) [38]. According to this, key variables to be optimized include: (a) tissue embedding; (b) cryosectioning; (c) matrix deposition; and (d) detection, as described below.

5.2. *Tissue embedding and cryosectioning*

Sample preparation begins with embedding the sample to facilitate sectioning. The most used embedding medium is 10 % gelatin in water

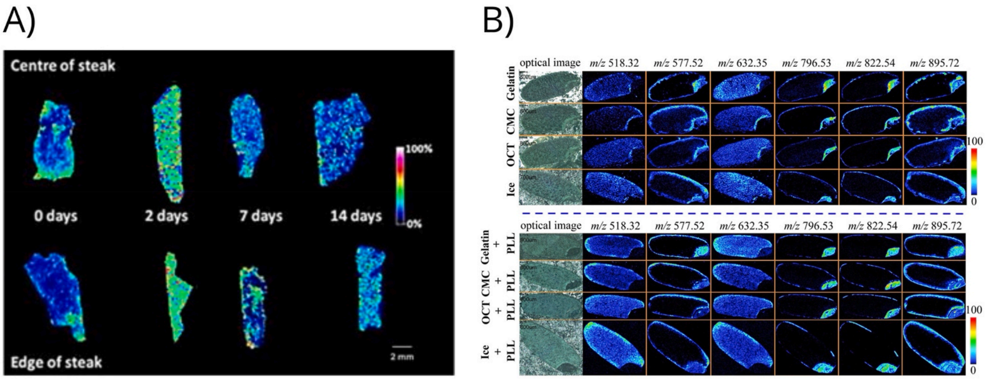

Fig. 4. Comparative MALDI-MSI image quality: (A) Distribution of lysophosphatidylcholine in steak sections at different storage times (200 μm spatial resolution), reprinted from Ref. [28]. (B) Rice grain section showing distinct lipid species under different sample preparation methods (35 × 35 μm spatial resolution), reprinted from Ref. [41].

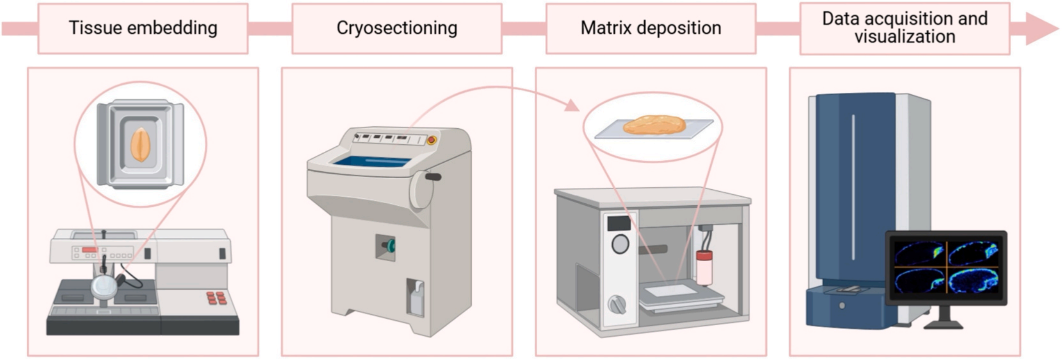

Fig. 5. Typical MALDI-MSI workflow from sample preparation to data acquisition.

(see Table 3). However, alternative materials such as 1 % carboxymethyl cellulose (CMC) solution, optimal cutting temperature compound (OCT), and ultrapure water have also been explored [38]. In comparative studies, gelatin was found to be the optimal option for rice matrices. Following embedding, the sample is transferred onto conductive glass, typically using indium tin oxide (ITO)-coated glass slides combined with conductive double-sided adhesive tape. This step requires caution, particularly for dry and fragile tissues, as direct embedding may cause cracking, ultimately resulting in reduced signal intensity, compromised accuracy, and consequently poor MALDI image quality [38]. Once mounted, the samples are frozen at −80 °C or lower, using a standard freezer, dry ice [62], or liquid nitrogen [60], and stored for several hours prior to cryosectioning.

Cryosectioning is a critical step in MALDI-MSI, essential for preserving the spatial integrity of the sample and ensuring accurate localization of target analytes. While this technique is well established for biological tissues such as organs, most standardized protocols have been developed with those soft, hydrated samples in mind. As a result, food matrices, especially hard or dry ones, are often overlooked or require specific adaptations. Zhang et al. [38] reported that conventional sample preparation methods are generally unsuitable for dry cereals due to their rigid cell walls and low moisture content, which frequently lead to shrinkage, fragmentation, and analyte delocalization or loss during sectioning. Therefore, this shows cryosectioning parameters must be carefully optimized for each specific matrix. One key factor in the optimization is the choice of slice thickness, as it plays a vital role in maintaining spatial accuracy and ensuring optimal sensitivity. Depending on the structural and compositional characteristics of the food sample, different section thicknesses may be required (see Table 3). This always must be done keeping in mind that the final objective is to balance mechanical integrity with analytical performance, preserving representative molecular distribution without causing further issues in terms of breaking the matrix or obscuring important signals. Zhang et al. [45] demonstrated this challenge in lentil samples by experimenting with section thicknesses ranging from 10 to 60 μm. They observed that thinner sections (10–15 μm) were prone to shrinkage and surface voids, which not only disrupted sample morphology but also weakened the ion abundance of some relevant molecular species. In contrast, thicker sections preserved the lentil tissue's structural integrity and enabled stronger signal intensities. A thickness of 45 μm was found to be optimal, allowing clear visualization of fine anatomical features such as the plumule in MALDI imaging. However, exceeding this thickness introduced insulating effects, hindering ion detection without significantly improving signal intensity. A similar evaluation was carried out for rice samples, in which section thicknesses ranging from 4 to 30 μm were tested [44]. It was observed that the signal intensities of five representative molecular ions were notably lower in the thinnest sections (4 and 8 μm), and the resulting images showed discontinuities and fragmentation. In contrast, sections with thicknesses between 12 and 30 μm exhibited progressively stronger signal intensities. On the other hand, when thickness exceeded 20 μm, the structural integrity of the tissue was compromised. These thicker sections became increasingly fragile, leading to significant fragmentation, localized contamination, and poor shape preservation, which in turn affected the accuracy of the imaging results.

This characteristic of MALDI-MSI, meaning the possibility of direct analysis of the sample without the need for extraction, positions it as a powerful alternative to traditional imaging techniques like immunochemistry or, in some instances, MALDI-MS for that matter. Unlike some immunological methods, MALDI-MSI bypasses labour-intensive protocols, avoids issues like antibody cross-reactivity, and eliminates the requirement for prior knowledge of target analytes [15]. It is also worth noting that this approach aligns with the principles of white analytical chemistry (WAC), as it can eliminate the need for extraction reagents [44,45,60,62] that are typically harmful to the environment (e.g. chloroform). Nonetheless, it can also be seen in the opposite light. Cryosectioning has a need for very low temperatures for quite long periods of time, depending on the waiting time for analysis besides the freezing itself. This makes the energy use way higher. In any case, it all depends on how strictly we consider each parameter, as it usually happens when speaking of Green Analytical Chemistry.

5.3. Matrix deposition & detection

In the reviewed literature, DHB is still the most used matrix for MSI, but the percentage is similar to CHCA and 9-AA, which makes sense given that negative mode is applied in a half of the reviewed studies related to the technique. DAN is also used as it offers high sensitivity, but it causes significant fragmentation and is both carcinogenic and mutagenic as well [16]. In MALDI-MSI, besides all the requirements named in MS, the application and crystal size can significantly influence both ionization efficiency and spatial resolution [4,61]. The matrix is no longer mixed with the sample or merely placed on it, but it must be applied to the whole section of the sample that is going to be analysed. The three main forms of matrix deposition in food matrices are spraying, airbrushing and sublimation (see Table 3). The spraying method consists of nebulizing the matrix solution onto the sample surface, typically using an automated device that moves at a constant speed to ensure even coverage. Parameters such as nozzle temperature, flow rate, air pressure, number of passes, and drying time are adjusted to obtain a homogeneous and sufficiently thick matrix layer. However, the solvent used in this method can occasionally cause analyte delocalization or uneven crystal formation if not optimized [64]. A similar method is airbrushing, which involves applying the matrix solution with a pressurized airbrush system. While the process is relatively simple and quick, it depends heavily on operator control, particularly in spray velocity and distance. This can lead to variability in matrix distribution and, if excessive solvent is applied, to potential diffusion of small molecules. Modifications to the method, such as incorporating a non-contact heating lamp, can help reduce such effects by improving drying and deposition uniformity [17]. Lastly, sublimation is a solvent-free method based on the direct phase transition of the solid matrix into vapor under vacuum. In this approach, the matrix is heated at reduced pressure which causes it to condense uniformly onto a cooled sample surface. This produces a consistent layer of fine crystals, preserving spatial integrity and minimizing diffusion, generally requiring less time than spraying. Nonetheless, due to the absence of solvent, the extraction efficiency for certain analytes may be lower unless a recrystallization or post-spray step is added [64].

Considerations involving the matrix deposition process were thoughtfully examined by Zhang et al. [38], who optimized different matrix deposition methods along with matrix solvent composition, matrix airbrushing concentration and matrix sublimation thicknesses. The matrix deposition methods included airbrushing, sublimation, and a two-step combination of both. In airbrushing, different concentrations of 9-AA, DHB, and CHCA were tested using methanol:water (70:30, v/v) with or without 0.1 % TFA and sprayed onto tissue sections using a nebulizer from a 10 cm distance, followed by vacuum drying. Sublimation involved depositing 300 mg of each matrix onto slides under controlled temperatures (180–250 °C) to achieve specific thicknesses ranging from 0.5 to 2.0 μm. Finally, the two-step method first applied the matrix via sublimation and then enhanced coverage by airbrushing. Microscopic imaging confirmed variations in crystal morphology depending on the deposition method, which evidences that different matrix deposition methods require distinct optimization parameters. For instance, Wang et al. [62] optimized a spraying approach by systematically evaluating variables such as spray nozzle temperature, flow rate, spray air pressure, number of passes, and drying time per cycle. In contrast, sublimation-based deposition typically involves a simpler optimization process, requiring only the control of temperature and time to achieve a desired matrix thickness, as was seen in the previous case as well [38].

6. Current challenges and future directions

Despite the utility of MALDI-MS and MALDI-MSI in lipid analysis, which has been demonstrated in the reviewed articles, several technical hurdles remain that limit their broader application, particularly in terms of accurate quantification. One of the most persistent issues is the ion suppression effect, which can significantly bias the detection of specific lipid classes. This variability is often influenced by the sample matrix or the use of embedding compounds (for MSI) such as OCT, which are known to interfere with ionization and reduce signal intensity for certain analytes [57]. To mitigate this issue, future developments could focus on alternative ionization strategies, including matrix-free laser desorption/ionization or the design of novel matrices with improved selectivity and reduced suppression effects. This could be further studied with new tools such as artificial intelligence and machine learning. The latter is starting to be applied in this area [24,51,65]. Another promising approach involves the use of internal standards and cross-platform data correction to improve the reliability of relative quantification across diverse sample types. Beyond the chemistry of ionization, advances are also expected in instrumentation and data processing. Enhancing spatial resolution, refining software tools for co-localization analysis, and integrating data from other “omics” could all contribute to a more comprehensive understanding of food chemistry. Furthermore, it is worth noting that none of the reviewed articles explicitly applied WAC metrics to evaluate their methodologies. This highlights a potential opportunity for future research, where incorporating such sustainability-focused criteria could help guide more environmentally conscious method development. MALDI-related techniques already offer promising features in terms of sustainability: they typically require very small amounts of sample and solvent and allow for rapid analysis with minimal sample preparation. These advantages suggest that MALDI-based workflows could also align well with WAC principles if consciously developed with that goal in mind. However, solvent usage, particularly of hazardous substances like chloroform, which is frequently employed in this technique, could be reconsidered.

7. Conclusions

Lipids serve as valuable biomarkers for elucidating biological and physicochemical processes in food, as well as for detecting food adulteration and fraud. Among the various analytical strategies available, MALDI-MS and MSI have proven to be particularly versatile tools, offering rapid analysis with minimal sample preparation in many cases. These techniques have already been employed to detect lipid species across all main categories Notably, GLs are the most frequently analysed lipid class and within this group, TAGs are the primary focus. Regarding matrix selection, DHB and super-DHB are the most applied in positive mode while, 9-AA is typically employed under negative mode conditions. Although applications of MALDI-MS in food science are less common in food than biological matrices, in this review, a wide variety of the former has been explored. Lastly, technical capabilities of the method have been improved, for example in terms of image resolution and analytical depth. Altogether, MALDI-MS represents a powerful and evolving platform for lipidomic investigations in the agri-food sector.

CRediT authorship contribution statement

Ana Jano: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **Adrián Fuente-Ballesteros:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **M. Teresa Martín:** Writing – review & editing, Visualization, Supervision. **Ana M. Ares:** Writing – review & editing, Visualization, Supervision, Methodology, Conceptualization. **José Bernal:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

9-AA, 9-aminoacridine; **AgTFA**, silver trifluoroacetate; **AMS**, ambient mass spectrometry; **CE**, cholesterol esters; **CHCA**, α-cyano-4-hydroxycinnamic acid; **CMC**, carboxymethyl cellulose; **DG**, diacylglycerols; **DGDGs**, digalactosyldiacylglycerols; **DHB**, 2,5-dihydroxybenzoic acid; **ESI**, electrospray ionization; **FA**, fatty acyls; **GL**, glycerolipids; **GP**, glycerophospholipids; **HRMS**, high-resolution mass spectrometry; **ITO**, indium tin oxide; **LIPID MAPS®**, LIPID Metabolites and Pathway Strategy; **LMSD**, LIPID MAPS® Structure Database; **MALDI-MS**, matrix-assisted laser desorption ionization mass spectrometry; **MALDI-MSI**, matrix-assisted laser desorption ionization mass spectrometry imaging; **MALDI-TOF MS**, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; **MS**, mass spectrometry; **OCT**, optimal cutting temperature compound; **PK**, polyketides; **PR**, prenol lipids; **PUFAs**, polyunsaturated fatty acids; **SL**, saccharolipids;

SP, sphingolipids; **SPE**, solid-phase extraction; **SQDGs**, sulfoquinovosyldiacylglycerols; **ST**, sterol lipids; **TAGs**, triacylglycerols; **TFA**, trifluoroacetic acid; **TOF**, time-of-flight; **UV**, ultraviolet; **WAC**, white analytical chemistry.

Data availability

The datasets generated during the current study are included in this published article, or they are available from the corresponding author on reasonable request.

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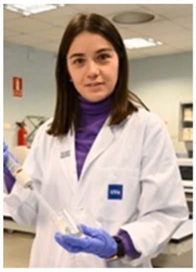

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