Application of mass spectrometry-based metabolomics approaches for food safety, quality and traceability.

María Castro-Puyana[1], Raquel Pérez-Míguez[1], Lidia Montero[2], Miguel Herrero[2]*

[1]Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Ctra. Madrid-Barcelona, Km. 33.600, 28871 Alcalá de Henares, Madrid, Spain

[2]Laboratory of Foodomics, Institute of Food Science Research (CIAL-CSIC), Nicolás Cabrera 9, Campus UAM Cantoblanco, 28049 Madrid, Spain

Corresponding Author: M. Herrero

e-mail: m.herrero@csic.es

TEL: +34 910 017 946

FAX: +34 910 017 905

ABSTRACT

The always more-demanding fields of food safety, quality and traceability are continuously fostering the development of robust, efficient, sensitive and cost-effective analytical methodologies. Mass spectrometry-based metabolomics is a key tool nowadays with great potential in many analytical fields and has been demonstrated to be capable of facing some important challenges related to these areas within the food science domain. The main aim of this review is to present a critical overview of the most recent applications of MS-based metabolomics approaches for food quality, safety and traceability assessment, covering the most relevant works published from 2014 to 2017. Information about the different steps needed to develop a MS-metabolomics approach, i.e. sample treatment, analytical platform, and data processing, is also provided and discussed.

Keywords: chemometrics, contaminants, food analysis, food quality, food safety, food traceability, foodomics, GC-MS, hyphenated techniques, LC-MS, mass spectrometry, metabolomics.

Abbreviations: CE, capillary electrophoresis; CID, collision-induced dissociation; DBDI, dielectric barrier discharge ionization; EVOO, extra virgin olive oil; GC, gas chromatography; GC × GC, comprehensive two-dimensional GC; HILIC, hydrophilic interaction chromatography; HRMS, high resolution mass spectrometry; HS-SPME, headspace solid-phase micro-extraction; ICP, inductively coupled plasma; IT, ion trap; LAESI, laser ablation electrospray ionization; LC, liquid chromatography; LC × LC, comprehensive two-dimensional liquid chromatography; LLE, Liquid-liquid extraction; MRL, maximum residue limit; MRM, multiple reaction monitoring; MS, mass spectrometry; MVOCs, microbial volatile organic compounds; NMR, nuclear magnetic resonance; PCA, principal components analysis; PLE, pressurized liquid extraction; PTR, proton transfer reaction; Q, quadrupole; QqQ, triple quadrupole; QTOF, quadrupole-time-of-flight; SLE, Solid-liquid extraction; SPE, solid-phase extraction; SPME, solid-phase microextraction; SRM, selected reaction monitoring; TOF, time-of-flight; UAE-DLLME, ultrasound-assisted extraction in tandem with dispersive liquid-liquid microextraction; UHPLC, ultra-high pressure liquid chromatography.

1. INTRODUCTION.

Metabolomics is one of the main branches in the field of the -omics techniques, and together with genomics, transcriptomics and proteomics, is involved in the study of the food and nutrition domains through Foodomics approaches. As per definition, metabolomics includes the exhaustive study of the whole small metabolite composition of a particular system or organism, understanding by small metabolite typically those with a molecular weight below 1500 Da. In practice, this aim is difficult to achieve, due to the huge chemical variability of metabolites that is often found; this implies that a universal approach to analyze using a single method metabolites belonging to very different chemical classes (significantly different polarity) as well as present in a very wide dynamic range is not attainable. In this regard, the food metabolome is not an exception as quite diverse compounds, such as carbohydrates, lipids, proteins, amino acids, amines, steroids, phenolic compounds, carotenoids, alkaloids or volatile compounds, among others are frequently present. For this reason, the selection of more than one analytical approach, and their combination for results interpretation is often carried out.

The analytical procedures usually employed within metabolomics can be grouped in different categories. On the one hand, methods can be classified under fingerprinting approaches or under profiling methodologies. Fingerprinting is referred to the analysis of as many compounds as possible within a system, including their detection and the subsequent statistical treatment of the obtained results in order to look for sample patterns. Under this approach, the identification and quantification of the detected metabolites may not be a necessity. In opposition, profiling refers mainly to the analysis of closely related metabolites, often belonging to the same chemical class, which are most frequently identified and quantified. Similarly, metabolomics approaches can be also classified as non-targeted or targeted analysis; whereas non-targeted approaches look for maximum

74 coverage of metabolites that can be simultaneously identified in a particular system,
75 targeted approaches are based on the determination and identification of a certain type of
76 metabolites, that could either belong to the same chemical class or being involved in a
77 particular pathway. In any case, as the complexity of the set of metabolites to be analyzed
78 is quite high in both approaches, suitable analytical techniques are needed, as well as
79 proper sample treatment methodologies. This latter subject is of great relevance in food
80 analysis, as food are usually quite complex matrices full of potentially-disturbing
81 components for the analysis of metabolites. Sample treatment may be relatively simple
82 or involve multiple steps. However, it has always to be considered that sample treatment
83 may include unintended bias towards the metabolites present, as a universal sample
84 treatment directed to the extraction of the full metabolome of a particular sample will not
85 exist in practice, and thus, some components may be lost during this phase.
86 Concerning the analytical tools employed, most attention has been paid to the detection
87 technique. However, it is evident that a proper separation before detection can increase
88 the quality of the obtained results. Although gas chromatography (GC) was perhaps the
89 separation technique of choice in the initial metabolomics studies, the need for
90 derivatization in order to increase the coverage of compounds that can be analyzed
91 following this approach has driven to shift the primary technique to liquid
92 chromatography (LC). In fact, LC can be operated in several separation modes, which
93 increases its versatility towards the separation of a variety of different metabolites.
94 Particularly, in the last years, methods based on the use of ultra-high performance liquid
95 chromatography (UHPLC) have gained considerable popularity thanks to the advantages
96 that this technique can provide with, including high efficiency, good resolution, relatively
97 short analysis times and the use of flow rates fully compatible with mass spectrometry
98 (MS) detection.

Likewise, concerning the detection of the metabolites, nuclear magnetic resonance (NMR) was the most-used technique in the first years of metabolomics development. However, MS has gradually substituted the use of NMR. Some of the reasons behind this move include that MS is by far more suitable for coupling with a separation technique, as well as the development and improved affordability of high resolution MS instruments. In this regard, the use of high resolution instruments, like time-of-flight (TOF) analyzers, or even hybrid instruments such as quadrupole-TOF (QTOF) or orbitrap, allows to obtain accurate mass determination, which is the key for their use in metabolomics approaches, as well as to resolve isomeric and isobaric species. Moreover, the possibility of running MS/MS experiments with some of these instruments, significantly enhances the capabilities for the identification of unknown metabolites.

As a direct consequence of the improvement on the available analytical tools, samples with higher complexity can be analyzed in which even thousands of features may be detected. Thus, the datasets generated after sample analyses in a typical metabolomics study is of extremely great complexity, including retention times, intensities, *m/z*, and even MS/MS spectra. Under these conditions, the manual interpretation and elaboration of all these data is impossible. For this reason, normalized procedures have been developed relying on bioinformatics tools in order to be able to properly extract the key information of all the huge amount of data available. Usually, data-processing involves peak detection, integration, peak alignment and normalization. After these steps, different chemometric tools can be used to statistically assess possible differences among samples. To do that, multivariate analysis is often used, although the particular statistical approach to be used will largely depend on the objectives of the study. Principal components analysis (PCA) is frequently employed at first, as it allows to group samples as a function of different variables. However, the particular statistical analyses made are usually

124 different depending also on the topic of the study, i.e., food-health relationships,
125 biomarker discovery, food quality, food safety or traceability, among others.
126 The aim of this review is to update the information provided in our previous article [1],
127 including a critical revision of the latest research published in the field of MS-based
128 metabolomics applied to food quality, food safety and traceability from 2014 to 2017. For
129 the sake of clarity, each of these three topics are described and discussed in separate
130 sections so that the basic particularities of the approaches involved in those subjects can
131 be appropriately described.
132

133 **2. MS-BASED METABOLOMICS FOR FOOD SAFETY**

134 Food safety is one of the most-important topics within food analysis; although one may
135 tend to consider that every sold and consumed foodstuff possess proper safety, the truth
136 is that food control is constantly required to maintain an appropriate degree of security
137 for consumers. Food safety involves many sub-fields, including the legislation
138 enforcement regarding the presence of selected compounds in foods that may be present
139 below certain limits (MRL, maximum residue limits), the detection of microbial-related
140 spoilage, the determination of allergens, the detection of environmental contaminants as
141 well as banned external compounds, or the assessment of the occurrence of natural toxins,
142 for example. In this regard, the use of MS within metabolomics-based approaches has
143 allowed significantly raising the level of the analytical determinations possible nowadays.
144 In this section, the most-relevant published procedures to this aim are described and
145 commented.
146

2.1. Detection of chemical contaminants: food production-related controlled substances (veterinary drug and pesticide residues), environmental pollutants and food-contact materials

Although there is a wealth of published material developing always better analytical methods for the detection of selected contaminants in foods, this section is focused to those methods that take advantage of metabolomics-based approaches to carry out those determinations, thus, targeting the detection of multiple components in just one run.

The first part of any MS-based metabolomics study for the detection of food contaminants is sample preparation. As foods may be considered as very complex matrices involving the presence of a broad array of very different components, suitable sample preparation steps are needed in order to allow a proper detection of contaminants which will surely be present in very low amounts. Some of the naturally present compounds in foods will negatively influence the analysis of the targeted compounds, and thus, different methods have been widely used to extract and/or concentrate those. Solid-liquid extraction (SLE) or liquid-liquid extraction (LLE), depending on the physical nature of the samples, using conventional solvents and solid-phase extraction (SPE) are, probably, the three sample preparation methods traditionally most-employed. However, following the latest trends regarding the application of "Green Chemistry" principles, other miniaturized protocols limiting the volumes of solvents employed have been also proposed and employed in the last years. Among them, solid-phase microextraction (SPME) [2], and most notably, QuEChERS methods are highlighted [3]. Nowadays, QuEChERS involves a widely accepted methodology for the recovery of target analytes from complex matrices, which is based on an initial extraction with acetonitrile followed by a clean-up using dispersive SPE [4]. From this basic methodology, multiple modifications have been presented so far; these are mainly related to an adaptation to the nature and fat content of the sample

extracted [3]. Other advanced extraction techniques, such as pressurized liquid extraction (PLE), have also been successfully employed. These environmentally green tools even allow the coupling with in-line clean-up steps using adsorbents. This strategy was followed for the extraction of pesticides from honey that were subsequently analyzed by GC-MS/MS [5]. Readers interested on gaining deeper insight on extraction methods and sample preparation for the analysis of contaminants in foods are referred to recent excellent review papers [2,6-12].

Methods directed to quantification of chemical contaminants in food are strongly influenced by current international legislation, which is generally directed to the establishment of MRLs on certain substances, and to specify the banned compounds that cannot be present at any concentration. MRLs for pesticides [13, 14], veterinary drugs [15, 16] and contaminants [17], are available.

The most frequent analytical approach to determine contaminants in foods relies on the use of tandem MS detection. This detection procedure allows the quantification of known compounds with great selectivity and sensitivity. Typically, triple quadrupole analyzers have been widely used to this aim, run under selected reaction monitoring (SRM), also called multiple reaction monitoring (MRM), mode. This way, each parent ion is fragmented by collision-induced dissociation (CID) and its two most-intense product ions are detected. The most-intense one is used for quantification whereas the second is employed for qualification purposes. This detection procedure allows complying with European legislation on banned and controlled substances in foods [18]. This regulation establishes the requirements that an analytical method must meet for an unequivocal identification and quantification of a controlled substance in a food sample, which means to gain, at least, four identification points. By using the mentioned approach, the legislation specifies that one identification point is gained by retention time confirmation

with a commercial standard, whereas additional 1.5 identification points are gained for each ion transition successfully confirmed. As a result, and thanks to the quite fast scanning speed of modern triple quads, different remarkable applications have been developed in this field. In Table 1, some recent examples of this methodology for the quantification of more than 50 contaminants in foods in just one run are summarized. As it can be observed, most applications are based on the coupling of MS with a separation technique. LC and GC-based methods are widely extended, although the use of multidimensional chromatography has also been explored with success. Multidimensional procedures allow increasing resolving power and separation which can be beneficial for subsequent MS-based detection, considering that the targeted compounds will reach the detector more separated in time. This is the case of comprehensive two-dimensional gas chromatography (GC × GC) that has been coupled to a TOF-MS analyzer to determine dioxin-related pollutants in complex food samples [54]. Satisfactory separation of more than 200 micropollutants was achieved, with low limits of detection. Figure 1 illustrates the good separation attainable using this approach. Although no practical application of comprehensive two-dimensional liquid chromatography (LC × LC) has been published so far for the quantification of a wide group of contaminants, the use of this technique retains a very good potential. In fact, a first application for the quantification of pesticides in complex food samples, such as wine, has recently been presented [62]. As can be deduced from the information presented in Table 1, during the period covered by the present review (2014-2017), the use of triple quadrupoles in MRM mode is still the most-extended approach. Satisfactory results have been attained in a variety of applications involving the use of these approaches, using targeted approaches and reaching the quantification of a significant amount of components in relatively short analysis times with high sensitivity. Although the basic

222 principles remain relatively constant, different modifications have pushed even forward
223 the limits of these procedures. This is the case, for instance, of the use of high resolution
224 MS (HRMS) analyzers instead of the commonly employed triple quads; in fact, the use
225 of HRMS in the field of food safety is showing an increase. For instance, thanks to the
226 use of nano-LC and HRMS coupled through the use of ambient dielectric barrier
227 discharge ionization (DBDI) source, extremely low detection limits, as low as 10 pg mL^{-1}
228 , were achieved for the quantification of pesticide residues [63]. In fact, one of the
229 possible advantages of using HRMS is the possibility of constructing databases for the
230 sought compounds, when operating under targeted approaches. The use of these databases
231 together with parallel reaction monitoring using a Q-Orbitrap analyzer has been shown to
232 be effective for the appropriate screening and quantification of 157 residues of different
233 nature in honey [42]. Similar approaches have involved an expansion on the studied
234 compounds to more than 600 different contaminants, including pesticides, veterinary drug
235 residues, contaminants, perfluoroalkyl substances, mycotoxins and nitrosamines [61]. In
236 any case, each MS detection method has its highs and lows; comparative studies testing
237 the performance of tandem MS versus HRMS to quantify polychlorinated dioxins and
238 biphenyls in foods have concluded that although the use of GC-MS/MS allows meeting
239 with the requirements laid by the European Commission, GC-HRMS may fit better for
240 monitoring purposes as it was shown to produce less false positives [64].
241 In spite of the developed methods, the use of the above described targeted approach has
242 important limitations, which are mainly related to the determination of unknown
243 compounds as well as the need of reference commercial standards. For this reason, the
244 use of similar approaches already developed in other fields for the non-targeted analysis
245 of contaminants is increasingly proposed, taking advantage of the capabilities of HRMS
246 modern analyzers [65]. An interesting example has recently been published in order to

247 investigate which compounds of potential concern were present in a pizza box, as a model
248 of food packaging material [26]. This approach involved the coupling with proper *in-vitro*
249 assays based on aryl hydrocarbon receptor activity to limit the number of fractions to be
250 studied after extraction. The most-active fractions were analyzed by using GC-QTOF-
251 MS and UHPLC-QTOF-MS. The workflow followed in this work is shown in Figure 2.
252 Seventy-five substances were tentatively identified, among which seven commercially
253 available could be further studied but could not explain a significant proportion of the
254 aryl hydrocarbon receptor response in the extract. Thus, it could be concluded that other
255 very active substances still remained unidentified in the food container [26]. Using
256 another different non-targeted approach Zomer and Mol also showed the high potential
257 of state-of-the-art HRMS instrumentation [50]. Using a hybrid HRMS analyzer, a new
258 fully non-targeted approach for data acquisition combining full-scan and fragmentation
259 was developed utilizing variable data-independent acquisition for the generation of
260 fragment ions. Quantitative validation of the methodology using a mixture of 184
261 pesticides in two food matrices showed that this approach was suitable for ca. 93 % of
262 the assayed pesticide/matrix/concentration combinations studied in agreement with EU
263 guidelines. Thus, this LC-full-scan HRMS method has been suggested as an alternative
264 for triple quad MS-based methods. Moreover, the same data could be used to screen
265 samples for a large number of compounds with lower probability of being present,
266 reducing the chance for false-negatives compared to other previously used full-scan-
267 based protocols [50].
268 The most interesting aspect related to the non-targeted methodology is based on the
269 possibility of detecting substances not previously pre-selected, thus, increasing the chance
270 for the proper detection of unknown and unexpected compounds. These metabolomics
271 approaches may gain advantage of data mining tools initially developed in other fields. A

proof-of-concept study, demonstrating the ability of these tools to identify unknown chlorinated chemicals in honey samples has been reported [29]. However, the use of these diverse non-targeted methodologies is still somewhat limited compared to the targeted approach, as it is clearly illustrated in Table 1. Further developments on this field in the near future are expected.

2.2. Detection of microbial contaminants (pathogens and toxins)

Risks of natural origin for food safety are mainly related to the presence or activity of microorganisms. Thus, foods may be contaminated directly by the presence of pathogens, which could cause an infection to the consumer, or may be indirectly contaminated by toxins produced by a particular microorganism. Contamination of food with pathogens may imply very serious consequences on health, being the most extended diarrhea, and can occur at any point of the food production chain due to inadequate hygiene conditions. On the other hand, the presence of toxin producers within or near food related products can be a potential source of contamination. This is the case, for instance, of cereal products contaminated with mycotoxins, or shellfish contaminated with microalgal toxins that are bioaccumulated in those filter-feeding animals.

For the detection and quantification of toxins in foods, similar approaches to those already described for chemical contaminants are widely employed. The methodology to quantify those components by tandem MS is very much the same; however, in this case, the natural toxin variability potentially present in a particular food product mean that less compounds have to be analyzed, and thus, advanced metabolomics-based approaches are not required. Instead, proper sample preparation for toxins extraction and quantification by MRM using triple quads is the most common MS-based methodology applied [66-67]. Nuts [68], maize [69], shellfish [70], tomato [71], or beer [72], among others, are examples of food

297 products assayed following this approach. However, some modifications have been also
298 introduced to this methodology in order to increase the performance of methods as well
299 as to allow a very sensitive detection, as some of the natural toxins that might be
300 potentially found in foods are very toxic (even lethal) at extremely low concentrations.
301 For instance, the use of a multiple antibody immunoaffinity column for the selective
302 extraction of 7 toxins before HPLC-MS/MS determination has been recently reported
303 [73]. This method allowed extending the linear range of the determination as well as to
304 decrease the detection limits to the low $\mu g\ kg^{-1}$ level compared to previously developed
305 methods. Other sample preparation-oriented improvements have been directed to the
306 implementation of inexpensive graphitized carbon for SPE of paralytic shellfish toxins,
307 showing excellent capabilities [74].
308 Other sensitive gains have been attained through the analytical tool employed prior MS.
309 The ultrasensitive detection, with detection limits as low as 0.38 fmol of saxitoxin was
310 achieved in seafood samples thanks to a reaction involving diethylenetriamine-
311 N,N,N',N'',N'''-pentaacetic acid. This compound can couple with saxitoxin and
312 simultaneously chelate with Eu^{3+} to allow metallic labeling of this toxin, that may be
313 quantified with extremely high sensitivity using capillary electrophoresis-inductively
314 coupled plasma-MS detection (CE-ICP-MS) [75]. Direct determination of toxins may
315 have the further advantage of increasing throughput in food safety laboratories. As
316 already mentioned, some direct analysis MS techniques have been employed for the
317 quantification of chemical contaminants (see Table 1). In the case of toxins, some direct
318 methods have been also presented. Indeed, domoic acid has been quantified in mussel
319 tissues directly by MS/MS using SRM mode without any sample extraction, clean-up or
320 separation. This has been obtained using laser ablation electrospray ionization (LAESI),
321 reaching limits of detection of 1 $mg\ kg^{-1}$ for this compounds. This LOD is not particularly

low compared to other more conventional approaches based on extraction/separation and MS/MS detection, but it has to be considered that each analysis takes around just 10 s, thus, being very attractive for routine analysis [76]. Although these recent advances have enhanced in different manners the detection of toxins in food, any of them shows a purely metabolomics-based strategy. In this regard, this subfield of analysis should benefit in the future from applications already developed for contaminants analysis as those previously described in Section 2.1. In spite from this, some efforts have already been made, such as the development of an analytical micro HPLC-MS/MS method for the simultaneous quantification of 26 mycotoxins in maize with total run times of 9 min and reduced solvent consumption (below 0.3 mL) [77].

Other food safety-related methodologies are mostly focused on the detection of pathogen microorganisms that could be present in the food products posing a serious risk to consumers' health. Although different molecular techniques and proteomics-based approaches may be used to detect and identify the microorganisms present in a sample, in recent years much effort has been also focused on the determination of microbial volatile organic compounds (MVOCs) as markers of microbiological contamination [78]. To that aim, the most-extended analytical MS-based approach is based on the use of GC-MS coupled to a proper sample preparation/extraction protocol, such as SPME or headspace (HS) sampling. After the determination of a group of volatiles as wide as possible, multivariate analysis of data is necessary to correlate the presence of specific compounds with the growth of particular pathogens. This approach has been employed to predict shelf-life, evaluating potential chemical spoilage indices of Atlantic salmon stored under aerobic conditions [79], sea bass stored under air and under modified atmosphere [80], sea bream depending on the storage conditions [81-82], as well as minced meat [83] or pork [84]. Another possibility gaining interest in recent times is the

347 determination of MVOCs by real time analysis through the application of proton-transfer-
348 reaction-MS (PTR-MS). This technique is able to provide with fast on-line analyses that
349 are very appropriate for determination of the real-time evolution of volatiles. Different
350 applications have been recently published to determine MVOCs of microbial origin from
351 selected strains [85] as well as in food products such as chicken meat [86] or milk [87-
352 88]. To allow the continuous on-line monitoring, different set-ups have been developed,
353 for instance, allowing the monitoring of four meat samples in parallel [86] (Figure 3A),
354 or other more manually-operated set-ups for milk (Figure 3B) [87].
355

356 **3. MS-BASED METABOLOMICS TO ASSESS FOOD QUALITY**

357 Nowadays, food quality is one of the major concerns of the food industry. Its evaluation
358 is a complex task due to the multiple aspects that may be considered to achieve an
359 appropriate food quality. Food composition, aroma, flavor, or nutritional properties are
360 among the most important aspects that may be evaluated in food quality assessments.
361 Different types of analysis are clearly needed to evaluate all these aspects. Is at this point
362 where MS-based metabolomics approaches are gaining attention due to their
363 demonstrated capability to establish links between relevant food aspects and food quality
364 perception.
365 Table 2 summarizes the most relevant applications of MS-based metabolomics strategies
366 for food quality published during the period of time covered by this review (2014-2017).
367 As can be observed, these works are mainly focused on the use of this kind of platform
368 to establish the relationship between the chemical composition and food quality, to
369 control food authentication and adulteration, or to differentiate food samples according
370 to their variety. To achieve these aims, non-targeted approaches have usually been
371 employed followed by data-processing and multivariate analysis to assess possible
372 differences among samples. An interesting strategy is the combination of non-targeted

373 and targeted methods; its usefulness has recently been reported for the qualitative analysis
374 of curcuminoids in turmeric [91]. This integrated strategy involves a non-targeted
375 analysis by LC-QTOF-MS/MS and a targeted approach by LC-QTRAP-MS/MS. Figure
376 4 depicts the workflow followed in this study. Ninety-six curcuminoids were fully
377 characterized following this exclusive methodology. Anyhow, the ultimate goal of the
378 researches developed to assess food quality is to determine relevant compounds that may
379 be selected as quality markers. Afterwards, just a few studies have developed targeted
380 methodologies for the routine analysis of those markers [89, 90]. However, this fact is
381 interesting from an analytical point of view, since a targeted method requires less
382 sophisticated instrumentation, is usually simpler and the data are more easily analyzed,
383 being, therefore, more applicable for routine analysis.
384 One of the relevant points to assess food quality by MS-based metabolomics is, again, the
385 choice of proper sample preparation procedures. This fact will depend not only on the
386 analytical technique employed to perform the analysis but also on the particular aim of
387 the study. Although nowadays the use of modern mass spectrometers enables to perform
388 analysis with high sensitivity which may simplify sample preparation, the inherent
389 complexity of food samples makes this step a critical factor in the determination of
390 metabolites, as previously mentioned. In any case, to prevent any substantial loss of
391 possible relevant metabolites, minimum sample preparation is preferable. Even though
392 simple solvent-based extraction procedures have been the method of choice during the
393 last years (see Table 2), certain GC-MS methodologies have required the use of other
394 sample preparation techniques such as ultrasound-assisted extraction in tandem with
395 dispersive liquid-liquid microextraction (UAE-DLLME) [98], solid-phase extraction
396 (SPE) [101], static headspace extraction (HS) [108] or headspace solid-phase micro-

extraction (HS-SPME) [115], in order to improve the extraction of volatile compounds or to achieve a preconcentration effect, thus, increasing method sensitivity and efficiency.

As can be deduced from the information shown in Table 2, the majority of applications of MS-based metabolomics approaches included the coupling LC-MS and/or GC-MS. Concerning LC-MS, the use of methods based on the UHPLC has increased considerably in the last years due to its capability to perform complex analysis with high efficiency and resolution in a short time. Different metabolomics studies have employed UHPLC technology for example to carry out the authentication and the evaluation of possible adulterations in fruits juices [89, 90] or saffron [99], demonstrating the feasibility of these methodology to face one of the most growing problems in the global market. Another point that should be highlighted regarding LC is that although C18 columns are by far the most utilized, methods based on the use of hydrophilic interaction chromatography (HILIC) have also successfully been applied to food quality. This allows profiling highly polar and hydrophilic compounds providing complementary metabolic information to reversed-phase LC. Even though there are some drawbacks associated with HILIC (variability in retention times, low peak efficiency, and long re-equilibration times after gradient elution), this methodology has been used for the assessment of contamination and degradation of infant formulas [97] or to identify biomarkers of meat quality [104, 106].

Regarding GC-MS, in spite of the need to include a derivatization step in the sample treatment to increase the range of metabolites that can be analyzed, GC-MS metabolomics approaches have been broadly used to evaluate food quality as it can be observed in Table 2. In these cases, GC has been hyphenated to a great variety of mass analyzers including simpler MS instruments, like quadruple (Q) working at electron ionization mode [98,102,103,112,115], or ion trap (IT) [114], as well as high resolution instruments

[93,95,105,109,110], and even hybrid analyzers [96,101,107]. An interesting work based on the use of GC coupled to TOF-MS has been employed to develop a non-targeted metabolomics approach capable to establish differences between wine grape cultivars [93]. To do that, two grape cultivars were profiled and 115 metabolites were identified and quantified. Among them, sugars and amino acids showed an opposite behavior in both cultivars. To carry out the biological interpretation of the data and to obtain an overview of the abundance of these compounds in the development of the cultivars, their behavior in the primary metabolism pathways was investigated. Figure 5 depicts the level of each metabolite within each cultivar during the grape development stage in different pathways (tricarboxylic acid cycle, glycolysis, amino acid synthesis, and sucrose synthesis). Other interesting strategies based on GC-MS metabolomics platforms have been applied, for instance, to investigate the effect of volatile compounds for the classification of saffron based on the concentration of biomarkers [98], to classify olive oils according to their quality parameters [101], or to detect milk or meat adulteration [103,107].

Although LC-MS and GC-MS have been the preferred platforms to assess food quality, GC × GC [108] and CE methods [104] coupled to TOF analyzers have also been applied with success. The first one has allowed to establish associations between volatile metabolites and perception of rice aroma, creating a panel of biomarkers of rice flavor quality [108]. These results are valuable for breeding programs since can be used to choose pleasant rice aromas. In the latter, the feasibility of using a polymer-coated-capillary for the separation of anionic metabolites both in orange juice and wine has been demonstrated [104]. It offers a complementary coverage of the metabolome of these samples to those provide by other analytical techniques. Due to the demonstrated capabilities of both GC × GC and CE, it is expected that future developments in this field

447 will gain advantage of those methods, since the full potential of these techniques in food
448 metabolomics has not been reached.
449
450 **4. MS-BASED METABOLOMICS FOR FOOD TRACEABILITY**
451 Food traceability is also a relevant topic within food analysis, whose main purpose is to
452 provide a continuous monitoring of a food in the entire supply chain; this monitoring has
453 been often defined as "from farm to fork". Undoubtedly, food traceability is closely
454 related to food quality, food safety and public health. This topic has a great importance
455 not only to food industries but also to consumers who are increasingly demanding more
456 information about each stage of the food that they consume. In this regard, MS-based
457 metabolomics approaches are essential since they are capable to provide the level of
458 accuracy needed for traceability management.
459 Bearing in mind that traceability involves knowing the composition and origin of a food,
460 it is clear that the determination of the geographical origin may be considered the starting
461 point for food traceability. Geographical origin assessments have not only relevant
462 implications from an economical point of view but also they are a key parameter in terms
463 of food quality. The most common metabolomics strategies developed to discriminate
464 food samples according to their geographical origin are non-targeted approaches based
465 on the use of LC (mainly UHPLC) coupled with HRMS. Using the most suitable sample
466 preparation protocols according to the features of each food sample and the appropriate
467 multivariate data analysis, these MS-based methodologies are able to point out different
468 metabolites as potential markers of food origin. This kind of approaches has successfully
469 been applied for the origin assessment of extra virgin olive oil (EVOO) [117] orange
470 [118], hazelnuts [119] or cocoa beans [120].
471 Other relevant branch in food traceability is focused on monitoring changes in the food

metabolic profiles produced by food-processing. Production steps, including for instance, heat treatments, fermentation, and storage, among others, can alter nutritional and organoleptic properties of foods, as well as lead to a substantial loss of health-promoting compounds. This fact has been demonstrated by a recent and interesting non-targeted UHPLC-QTOF-MS method developed to evaluate the phenolic profiles of three different processed tomato products and tomato paste produced by three different treatments [121]. The combination of the results obtained from the metabolomics analysis with total phenolic and lycopene content, and antioxidant capacity showed that processing affects the nutritional and health-promoting potential of tomato products. Besides, the metabolomics approach shows its high potential in traceability purposes since the treatment provide a characteristic phenolic profile.

Other non-targeted LC-HRMS platforms have also been applied with success to study the effect of storage conditions on the metabolic profile of red wine [122] or iceberg lettuce [123], as well as to compare the effects of thermal processing on *Brassica* vegetables [124]. After processing and carrying out the multivariate data analysis, the final purpose of this kind of studies is to find the relationship between the changes on the metabolite profile with a loss of food quality. Figure 6 shows an example of the data analysis procedure followed to explore the metabolome of lettuce in order to evaluate changes related to storage time and genetics. Fermentation and ripening are also relevant process which may change the food metabolome. Two interesting examples have been described in the literature to explore the changes that occur in the metabolic profile of cocoa beans [125] and cheese [126] as a consequence of fermentation and ripening process, respectively. Bearing in mind that these two processes are critical steps in the processing of high quality cocoa beans or in the formation of specific characteristics of cheese, the results obtained in these metabolomics assays are of high value for the food industry since

497 they shed new light into fermentation and ripening optimization.

498 Even though most applications developed for food traceability in the period of time
499 covered by this review are based on the coupling of MS with LC, GC-MS methodologies
500 have also been proposed. For instance, using headspace GC-MS non-targeted approach
501 was possible to distinguish the effect of different process steps (including not only thermal
502 processing but also blanching and high hydrostatic pressure) on the chemical composition
503 of mango [127]. Once again, the results obtained clearly demonstrate the influence of
504 these steps on the volatile profiles of processed products. GC-MS metabolomics approach
505 has also proven to be an excellent tool to evaluate the modifications that may occur during
506 the cooking of different types of pasta [128].

507 Another possibility gaining interest in recent times is the use of CE coupled to MS as
508 analytical platform for traceability assays. For example, Sugimoto et al. developed two
509 CE-TOF-MS methodologies for anionic and cationic metabolite analysis of dry-cured
510 ham [129]. The results obtained enabled to establish a correlation between the metabolite
511 profiles of twelve kinds produced in different countries and processed under different
512 conditions and the ripening period and processing conditions. Even though CE-MS
513 strategies are being mainly developed and applied for biological samples, nowadays, is
514 possible to find some applications devoted to food analysis. Further progress in this field
515 are expected in the near future.

516 Although non-targeted strategies have been the most-extended approach to evaluate
517 changes in the metabolic profiles of food samples during food-processing, targeted
518 analysis may also be very useful; this kind of approaches has been employed to evaluate
519 the metabolic changes that take place in two starch potato genotypes in response to
520 osmotic stress [130] or during avocado development and maturation [131].

521

5. CONCLUSION AND FUTURE OUTLOOKS

As it can be deduced from the update shown in this review paper, the use of MS-based approaches for food safety, food quality and traceability is still far from reaching its maximum potential. It is quite obvious that the use of MS, particularly high resolution MS, will still be dominant in studies on the mentioned fields in the years to come. In this regard, the continuous improvement of available instruments will be translated to enhanced capabilities of the developed methods. As MS is most frequently used hyphenated to other analytical tools, the improvement on robustness of couplings and available interfaces and ionization tools, including those employed in direct analysis, will positively influence the obtainable results. This way, new to-be-controlled substances appearing in the market as well as unknown ways to perform frauds during production of valuable food products could be discovered. Specifically, within the food safety field, new multi-residue and multi-targeted methods will surely continue appearing, ready to help on the food control area. However, more interestingly, the development of novel non-targeted metabolomics-based approaches will help to gain a holistic view of the food safety issue. Those procedures are clearly more capable of discovering new safety hazards beyond the use of the regulated compounds and contaminants. But those approaches could have even more potential if accompanied by proper in-vitro and in-vivo assays, so that the perspectives may be further opened, for instance, to the discovery of markers of toxicity.

Food quality will also benefit from the extension of metabolomics MS-based approaches to other studies. Within this field, the further application and development of these methodologies could help to increase the available knowledge on which compounds present in food that may have a still concealed importance for food quality perception. This is the case, for example, of the application of this kind of procedure to reveal the

whole sensory pattern of a food product, a concept already applied in flavoromics researches. Likewise, as metabolomics methods evolve in the future, new relationships between food components and particular characteristics related to food quality will be discovered.

Regarding traceability, much effort is expected to be focused on the development of new methodologies to assess food authentication and geographical origin of valuable food products. However, this field is intimately linked to food quality as some traceability aspects are related to quality. For instance, development of traceability potential will help to discover how production processes throughout the food production and commercialization chain may affect quality parameters. In this regard, the use of alternative analytical techniques to LC and GC, such as CE or multidimensional approaches (including LC × LC and GC × GC) could offer complementary selectivity and thus, information, that would help to increase the metabolite coverage of the studied system. This enhanced coverage could positively influence the applicability of MS-based metabolomics studies in the three different mentioned fields.

In summary, it is clear that although the interest of using MS-based metabolomics approaches in food safety, quality and traceability is already high, further developments in these methodologies will have a great influence on the mentioned fields in the near future.

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FIGURE CAPTIONS.

Figure 1. GC×GC-TOF/MS contour plot of the 209 PCBs and 17 PCDD/Fs with the Rtx-Dioxin2/BP-X50 column set. Adapted with permission from [54].

Figure 2. Workflow for the identification of compounds in fractions from pizza packaging material analyzed by GC-EI-qTOF MS and UHPLC-ESI-qTOF MS. Reproduced with permission from [26].

Figure 3. Schematic set-ups for continuous on-line monitoring of microbial volatile organic compounds by proton-transfer-reaction MS in A) the headspace of four meat samples in parallel (adapted with permission from [86]) and, B) in the headspace of milk samples (adapted with permission from [87]).

Figure 4. Workflow for establishment of curcuminoid profile in turmeric by an integrated strategy. Reproduced with permission from [101].

Figure 5. Scheme of the primary metabolism pathways of metabolites in Cabernet Sauvignon (CS) and Merlot (ME) cultivars during different grape development stage. Pathways are simplified version of tricarboxylic acid cycle, glycolysis, amino acid and sucrose synthesis. FLW, flowering; FS, fruit setting; PRV, pre-veraison; VR, veraison; PSV, post-veraison; RP, ripening. Metabolite intensity is color coded. Reproduced with permission from [93].

Figure 6. Data analysis workflow to explore the metabolome of lettuce in order to evaluate changes related to storage time and genetics. FB fast-browning cultivar, SB slow-browning cultivar, d0 day 0, d5 day 5. Reproduced with permission from [123].

1039 **Table 1.** Selected remarkable applications published during the period 2014-2017 dealing with the simultaneous identification and quantification

1040 of a large number of contaminants (> 50) in food samples.

Contaminants quantified	Food matrix	Sample preparation	MS-based approach	MS-based technique	Sensitivity		Reference
					LOD	LOQ	
Pesticides (54)	Fruits and fish	QuEChERS	Targeted	UHPLC-HRMS (Orbitrap)	< 2 ng mL^{-1}		19
Pesticides (54)	Tomatoes, oranges	QuEChERS	Non-targeted	GC-EI-HRMS (Orbitrap)		10 µg kg^{-1}	20
Pesticides (55)	Bivalves (*Scrobicularia plana*)	QuEChERS	Targeted	GC-MS/MS (IT in SIM mode)		0.33-10.3 µg L^{-1}	21
Pesticides (57)	Tomato	QuEChERS	Targeted	LC-MS/MS (QqQ in MRM mode)	< 5000 µg kg^{-1}		22
Antibiotics (62)	Meat	Solvent-based extraction (ACN)	Targeted	LC-HRMS (Orbirtap)	1 µg kg^{-1}	3.3 µg kg^{-1}	23
Contaminants (68)	Food contact materials	QuEChERS (modified)	Targeted	LC-MS/MS (QqQ in MRM mode) GC-MS/MS (QqQ in MRM mode)		1.3 – 220 µg kg^{-1}	24
Pesticides (73)	Fruits, vegetables	Solvent-based extraction (ACN)	Targeted	LC-MS/MS (QqQ in MRM mode)		< 10 µg kg^{-1}	25
Contaminants (75)	Food contact materials	Soxhlet-based protocol	Non-targeted / Targeted	UPLC-HRMS (QTOF, database)	< 2 ng ml^{-1}	< 20 ng ml^{-1}	26
Herbicides (76) and veterinary drug residues	Shellfish	QuEChERS	Targeted	LC-MS/MS (QqQ in MRM mode) GC-MS/MS (QqQ in MRM mode)		0.25-0.50 µg kg^{-1} veterinary residues 2-20 µg kg^{-1} pesticides	27
Veterinary drug residues (76)	Meat	Solvent-based extraction (ACN)	Targeted	UHPLC-MS/MS (QqQ in SRM mode)		0.038- 74 µg kg^{-1}	28
Pesticides and antibiotics (83)	Honey	Solvent-based extraction (ACN)	Targeted / Non-targeted	LC-HRMS (Orbirtap)	< MRLs		29
Pesticides (87)	Groundnut oil	QuEChERS	Targeted	LC-MS/MS		4 - 180 µg kg^{-1}	30

				(QqQ MRM mode)			
Pesticides (79) and antibiotics (13)	Honey	Solvent-based extraction (ACN) and clean-up	Targeted	UHPLC-MS/MS (QqQ in MRM mode)	0.03 to 1.51 µg kg−1	0.1 to 5 µg kg^{-1}	31
Pesticides (103)	Chicken, fish	QuEChERS	Targeted	LC-MS/MS (QqQ in dynamic MRM mode)		1-10 µg kg^{-1}	32
Pesticides (109)	Tomatoes	QuEChERS	Targeted	LC-MS/MS (QqQ in MRM mode)	0.5-10.8 µg kg^{-1}	1.3-30.4 µg kg^{-1}	33
Pesticides (113)	Rice, red pepper, mandarin	QuEChERS	Targeted	GC-MS/MS (QqQ in MRM mode)		0.1–25 µg kg^{-1}	34
Pesticides (115)	Oranges	QuEChERS	Targeted	LC-MS/MS (QqQ in MRM mode)	1 – 11 µg kg^{-1}	2 – 30 µg kg^{-1}	35
Pesticides (65) and environmental contaminants (52)	Kale, salmon, pork, avocado	QuEChERS	Targeted	GC-MS/MS (QqQ in MRM mode)			36
Pesticides (120)	Fruits, cereals	QuEChERS	Targeted	GC-MS/MS (QqQ in MRM mode)	10 µg kg^{-1}		37
Pesticides (120)	Apples, cucumbers	QuEChERS	Targeted	LC-MS/MS (QqQ in SRM mode)	1.2 – 11 µg kg^{-1}	10 µg kg^{-1}	38
Veterinary drugs (120)	Meat, eggs, milk	Ultrasound-assisted extraction and SPE	Targeted	LC-MS/MS (QqQ in MRM mode)	0.5–3.0 µg kg^{-1}	1.5–10.0 µg kg^{-1}	39
Contaminants (120)	Eggs	Solvent-based extraction (ACN) and purification	Targeted	LC-MS/MS (QqQ in MRM mode)		2.04–1316 µg kg^{-1} (CCβ)	40
PCBs (127), polychlorinated naphtalenes (6), PAHs (16)	Mussels, clams	PLE (100ºC, dichloromethane:hexane)	Targeted	GC-MS (quadrupole, SIM)		0.2-15 pg	41
Pesticides (105), antibiotics (49) and steroids (3)	Honey	Solvent-based extraction (ACN)	Targeted	UHPLC-HRMS (Orbitrap, in PRM mode and database)		0.009 - 6.21 µg kg^{-1} (CCβ)	42
Pesticides (133), PAHs (24)	Fish	QuEChERS	Targeted	GC-HRMS (QTOF)	10 µg kg^{-1}		43
Pesticides (162)	Tea	Solvent-based extraction (ACN) and purification	Targeted	GC-MS/MS (QqQ in MRM mode)	< 10 µg kg^{-1}		44

Pesticides (164)	Apples, broccoli, oranges	Polyurethane foam disks swabbing	Targeted	DART-HRMS (Orbitrap)	10 µg kg^{-1}		45
Pesticides (167)	Honey	Solvent-based extraction (ethyl acetate)	Targeted	LC-MS/MS (QqQ in MRM mode)		10 – 100 µg kg^{-1}	46
Pesticides (172)	Wines	Solvent-based extraction (ethyl acetate)	Targeted	LC-MS/MS (QqQ in MRM mode)		10 – 50 µg kg^{-1}	47
Pesticides (177)	Soy-based products	QuEChERS	Targeted	GC-MS/MS (QqQ in MRM mode)	0.1 - 10 µg kg^{-1}	0.5-20 µg kg^{-1}	48
Pesticides (178)	Eggs	Matrix solid-phase dispersion	Targeted	LC-MS/MS (QqQ in MRM mode) GC-MS/MS (QqQ in MRM mode)		5 – 10 µg kg^{-1}	49
Pesticides (184)	Lettuce, oranges	QuEChERS	Non-targeted	LC-HRMS (Orbitrap)	10 µg kg^{-1} (SDL) for 134 compounds 50-200 µg kg^{-1} (SDL) for 39 compounds		50
Pesticides (200)	Green lettuce, orange	Ultra-Turrax homogenization with methanol and dilution	Targeted	UHPLC-MS/MS (QqQ in MRM mode)		1.0 – 5.0 µg kg^{-1}	51
Pesticides (200)	Honey	QuEChERS	Targeted	GC-MS/MS (QqQ in MRM mode)	1.00 to 3.00 ng mL^{-1}		52
Veterinary drug residues (>200)	Milk	Solvent-based extraction (ACN)	Targeted	LC-HRMS (QTOF)	< 100 ng mL^{-1} (for 72% of compounds)		53
Dioxin-like micropollutants (206)	Meat	PLE (100 ºC, hexane)	Targeted	GC×GC-TOF/MS	0.050-0.100 µg kg^{-1} PCBs 65-227 ng kg^{-1} PCDD/Fs		54
Pesticides (219)	Cereals	QuEChERS	Targeted	GC-MS/MS (QqQ in MRM mode)		5 - 50 µg kg^{-1}	55
Pesticides (238)	Cabbage, cucumber	QuEChERS	Targeted	LC-MS/MS (QqQ in MRM mode)	0.02 - 6.32 µg kg^{-1}	0.06 – 21.06 µg kg^{-1}	56

Pesticides (269)	Avocado, citrus	QuEChERS with automated zirconia-based SPE	Targeted	LC-MS/MS (QqQ MRM mode)	< MRLs		57
Pesticides (317)	Vegetables, fruits	SPE	Targeted	LC-HRMS (QTOF and database)	10 µg kg^{-1} (84 %)		58
Pesticides (451)	Fruits, vegetables	QuEChERS	Non-targeted	LC-HRMS (Orbitrap)		< 5 µg kg^{-1} (85% of compounds)	59
Contaminants (492)	Milk, meat, eggs, liver, kidney, fish	Solvent-based extraction	Targeted	HPLC-HRMS (TOF-MS)	0.0005–100 ng mL^{-1}	0.003–250 ng mL^{-1}	60
Multiclass contaminants (625)	Baby foods, oranges, tomato	Solvent-based extraction (ACN)	Targeted	UHPLC-HRMS (QTOF and database)		< MRLs (excepting ca. 10% analytes)	61

ACN, acetonitrile; CCβ, detection capability; DART, direct analysis in real time; HRMS, high resolution mass spectrometry; IT, ion trap; MRL, maximum residue limit; MRM, multiple reaction monitoring; PAH, polycyclic aromatic hydrocarbons; PCBs, polychlorinated biphenyls; PCDD, polychlorinated dibenzo-p-dioxins; PLE, pressurized liquid extraction; PRM, parallel reaction monitoring; QqQ, triple quadrupole; SDL, screening detection limit; SIM, selected ion monitoring; SPE, solid phase extraction; SRM, selected reaction monitoring; TOF: time-of-flight.

1045 **Table 2.** The most remarkable MS-based metabolomics approaches devoted to food quality published during the period 2014-2017.

Food matrix	Metabolites	Sample preparation	MS-based approach	MS-based technique	Application	References
Pineapple, orange, apple, clementine, pomelo, and grapefruit juices	Flavonoids and limonoid glucosides	Centrifugation and filtering	Non-targeted / Targeted	UHPLC-HRMS (QTOF)	Detection of fruit juice adulteration	89
Citrus fruits, Jaffa, Mosambi orange and Red blush grapefruit	Flavonoids and limonoid glucosides	Centrifugation and filtering	Non-targeted / Targeted	UHPLC-HRMS (QTOF) for non-targeted LC-MS/MS (QqQ in MRM mode) for targeted	Discrimination of authentic and adulterated citrus fruits/fruit juices	90
Tumeric	Curcuminoids	Solvent-based extraction (using mixtures methanol:water)	Non-targeted/ Targeted	LC-HRMS (QTOF) for non-targeted LC-QTRAP-MS/MS (MRM mode) for targeted	Quality evaluation of raw turmeric from different regions	91
Grapes	Phytosterols	Solvent-based extraction (chloroform: methanol 1:1 (v/v))	Targeted	LC-HRMS (QTOF)	Discrimination of grapes according to plant sterols content	92
	Amino acids, fatty acids, acids (aromatic acids, hydroxy acids, dicar-boxylic acids, phenylpropanoic acids), flavonoid, and sugars	Solvent-based extraction (water: methanol:chloroform (1:2.5:1, (v/v/v))	Non-targeted	GC-HRMS (TOF)	Differentiation of cultivars through their metabolite profile	93
Graciano *Vitis vinifera* wine	Non-volatiles/ semivolatile metabolites (sugars, amino acids, higher alcohol, biogenic amines, organic acids and phenolic compounds)	Centrifugation and filtering	Non-targeted	LC-HRMS (QTOF)	Analysis of the metabolome of the Graciano *Vitis vinifera* wine variety	94

Tropical fruits (Mango, pineapple, jackfruit, baobab, tamarind)	Non-volatiles metabolites (carbohydrates, organic acids, amino acids, and fatty acids).	Solvent-based extraction (water), acid hydrolysis and derivatization with trimethylsilyl cyanide	Non-Targeted	GC-HRMS (TOF)	Comparison of non-volatile metabolites of tropical fruits	95
Soybean sprouts	Amino acids, organic acids, lipids, sugars, phytosterol, isoflavones, and soyasaponins.	Solvent-based extraction (50 % methanol for UHPLC; 50 % methanol followed by methoxylation, and derivatization with BSTFA for GC analysis)	Non-targeted	GC-MS/MS (QqQ in MRM mode), and UHPLC-HRMS (QTOF)	Evaluation of the relationship between germination and nutritional quality	96
Infant formulas	Low-molecular-weight compounds (nicotinic acid and nicotinamide were identified)	Solvent-based extraction (water) and ultrafiltration	Non-targeted	HILIC-HRMS (QTOF)	Assessment of contamination and degradation of infant formulas	97
Saffron	Volatile metabolites	UASE-DLLME	Non-targeted	GC-MS (Q with EI)	Investigation of the effect of volatile components on the saffron´s classification	98
	Glycerophospholipids and their oxidized lipids	Solvent-based extraction (ethanol:water 70:30 v/v) with sonication	Non-targeted	UHPLC-HRMS (QTOF)	Authentication of saffron	99
	Mainly flavonols and anthocyanins	Solvent-based extraction (ethanol:borate buffer at pH 9.0, 50:50 v/v) with sonication	Non-targeted	LC-HRMS (QTOF)	Investigation of the quality and authenticity of saffron	100
Olive oil	Volatile organic compounds	SPE	Non-targeted	GC-HRMS (QTOF)	Classification of olive oils according to their quality	101
Vinegar	Amino acids, carboxylic acids, sugars, sugar alcohols, fatty acids, vitamin, peptides and aroma compounds	MCF derivatization/TMS derivatization/ or extraction with diethyl ether	Non-targeted	GC-MS (Q with EI)	Comprehensive metabolite profile of vinegar	102
Milk	Short-chain hydroxylated carboxylic acids, long-chain stearic and palmitic acids, free amino acids, and sugars	Solvent-based extraction (methanol:chloroform) and derivatization with pyridine	Non-targeted	GC-MS (Q with EI)	Discrimination between milk typologies and detection of milk fraud	103

Orange juice and red wine	Mainly sugars, amino acids, and organic acids	Filtering	Non-targeted	CE-HRMS (TOF)	Comprehensive anionic metabolite profile of orange juice and red wine	104
Meat	Organic acids, amino acids, sugars, sugar alcohols, phosphorylated intermediates and lipophilic compounds	Solvent-based extraction (methanol:water 80:20 (v/v)) Derivatization with MSTFA for GC analysis	Non-targeted	GC-HRMS (TOF)/HILIC-HRMS (QTOF)	Identify biomarkers of meat quality traits	105
	Amino acids, sugars, nucleotides, nucleosides, and organic acids	Solvent-based extraction (methanol:water 80:20 (v/v) followed by chloroform:water 67:33 (v/v))	Non-targeted	HILIC-HRMS (Orbitrap)	Study of colour stability of ovine meat	106
	Amino acids, organic acids, alkane hydrocarbon, and sugar alcohols,	Solvent-based extraction (chloroform:methanol:water) and derivatization with MSTFA	Non-targeted	GC-HRMS (TOF)	Detection of the adulteration of beef meat	107
Rice (Jasmine phenotype)	Volatile organic compounds	Static HS extraction	Non-targeted	GC×GC-TOF/MS	Determination of the metabolites that define the 'Jasmine' quality of rice	108
Gochujang (fermented pepper paste)	Amino acids, organic acids, fatty acids, sugars, sugar alcohols, flavonoids, capsaicinoids, capsinoids, lipids	Solvent-based extraction (80 % methanol) Derivatization with MSTFA for GC analysis	Non-targeted	GC-HRMS (TOF)/ UHPLC-IT-MS	Quality characterization	109
	Mainly amino acids, organic acids, and sugars	Solvent-based extraction (80 % methanol) Derivatization with MSTFA for GC analysis	Non-targeted	GC-HRMS (TOF)/ UHPLC-HRMS (QTOF)	Evaluation of the metabolite differences according to the raw material used in the production of gochujangs	110
Green tea	Mainly catechins, amino acids, caffeine	Solvent-based extraction (hot water)	Non-targeted	UHPLC-HRMS (QTOF)	Study of the chemical composition of green tea to assess it quality	111
Peach fruit	Sugars, organic acids, and amino acids	Solvent-based extraction (methanol) and derivatization with MSTFA	Non-targeted	GC-MS (Q with EI)	Explore the chemical composition which defines fruit quality	112

Strawberry	Phenolic acids, flavonoids, flavan-3-ol derivatives, terpenes, and many types of glycosidically bound aroma and flavour precursors	Solvent-based extraction (80 % methanol)	Non-targeted	LC-HRMS (QTOF)	Separation and identification of major metabolites showing significant variation between strawberry cultivars	113
	Sugars, organic acids, and amino acids	Solvent-based extraction (methanol:water 1:1 (v/v)) and derivatization with MSTFA	Non-targeted	GC-MS (IT)	Differentiation of strawberry cultivars and assessment of the influence of agronomic conditions	114
Date palm fruit	Volatile metabolites (lipid-derived volatiles, phenylpropanoid derivatives, amino acid derived volatiles, and sugar derived volatiles)	HS-SPME	Non-targeted	GC-MS (Q with EI)	Differentiation among date varieties	115
Honey	Not described	Solvent-based extraction (methanol:water 1:1 (v/v) containing 1 % formic acid)	Non-targeted	UHPLC-HRMS (QTOF)	Discrimination of honeys according to their floral origin	116

BSTFA, bis(trimethylsilyl)trifluoroacetamide; DLLME, dispersive liquid-liquid microextraction; EI, electron ionization; HILIC, hydrophilic interaction liquid chromatography; HRMS, high resolution mass spectrometry; HS-SPME, headspace solid-phase micro-extraction; IT, ion trap, MCF, Methylchloroformate; MRM, multiple reaction monitoring; MSD, mass selective detector; MSTFA, N-Methyl-N-(trimethylsilyl) trifluoroacetamide; Q, quadrupole; QqQ, triple quadrupole; QTOF, quadrupole-time-of-flight; QTRAP, hybrid triple-quadrupole linear ion trap; SPE, solid-phase extraction; TMS, trimethyl Silyl; TOF, time-of-flight; UASE-DLLME, ultrasound-assisted solvent extraction in tandem with dispersive liquid–liquid microextraction.