Contributions of Chromatography to the Science Progress

Subjects: Chemistry, Analytical

Contributor: José Sousa Câmara , Cátia Martins , Jorge Pereira , Rosa Perestrelo , Silvia Rocha

Chromatography was born approximately one century ago and has undergone outstanding technological improvements in innovation, research, and development since then that has made it fundamental to advances in knowledge at different levels, with a relevant impact on the well-being and health of individuals. Chromatography boosted a comprehensive and deeper understanding of the complexity and diversity of human–environment interactions and systems, how these interactions affect our life, and the several societal challenges currently facing, namely those related to the sustainability of our planet and the future generations. From the life sciences, which allowed to identify endogenous metabolites relevant to disease mechanisms, to the OMICS field, nanotechnology, clinical and forensic analysis, drug discovery, environment, and “foodprint”, among others, the wide range of applications of today’s chromatographic techniques is impressive. This is fueled by a great variability of powerful chromatographic instruments currently available, with very high sensitivity, resolution, and identification capacity, that provide a strong basis for an analytical platform able to support the challenging demands of the postgenomic and post COVID-19 eras.

gas chromatography liquid chromatography science advances societal challenges

1. Introduction

Science has had a significant impact on the understanding of various phenomena associated with the evolution of humanity supported by a remarkable and unique technological evolution, namely from the fourth industrial revolution (4IR), which boosted the use of the internet of things (IoT), modern smart technology, and large-scale machine-to-machine communication (M2M). Chromatography also played a pivotal role in this accelerated development, regularly providing with new technical and technological advances that boosted the advancement of various areas of science in the post-genomics era, from clinical to agricultural and food sciences, including pharmaceuticals, environment, and health sciences, namely in the diagnosis of various pathologies, in the assessment of therapeutic efficacy and progression of the individual’s state, and, more recently, in the metabolomics field (**Figure 1**).

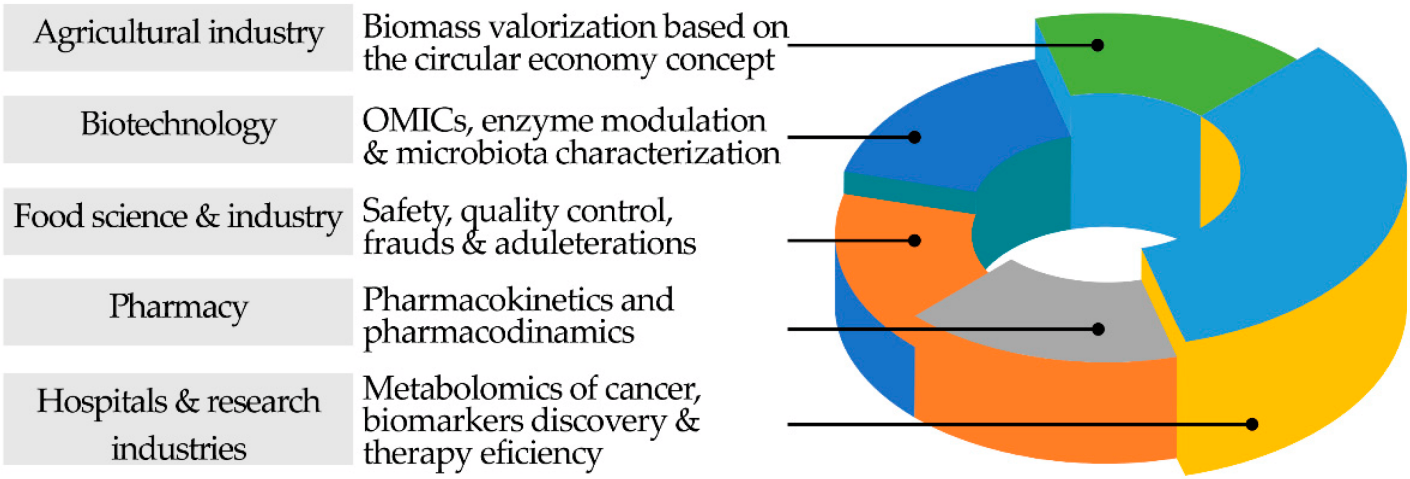

Figure 1. Important applications fields of chromatography in science development.

It has indeed had a major impact on the evolution of science, and it will also be important to create resilient innovation pathways. It allows to have a healthier, cleaner, and wealthier world. Its robustness and very high-resolution power associated, namely, with the most recent instruments, help to better understand and look for the building blocks of life, the Universe, and the possibility of life on other planets, which is the reason why chromatographic systems have been sent on different space missions such as Apollo (moon), Cassini–Huygens (Saturn), Rosseta (comets), and Viking and Curiosity (Mars) [1][2][3].

In fact, chromatography has a strong impact on our everyday life and its contribution to the progress of science is unquestionable, namely, when hyphenated with MS and other high-resolution analytical techniques. The technologic chromatographic advances in terms of columns and detection systems, made it possible to detect and quantify trace amounts of compounds affecting the very basics of life processes.

Currently, the usage of chromatographic techniques is mainly driven by the hospitals and R&D laboratories, the agricultural and food industry, pharmaceutical and biotechnology, the environmental industry, and the petrochemical industry (**Figure 1**). Much of the advances and progress in health sciences and medicine are directly related to technological advances in chromatography and related separation techniques. The availability of equipment with high-resolution power, sensitivity, and identification made possible a more comprehensive and in-depth knowledge of the metabolic alterations of our organism that allow to differentiate pathological from healthy states [4][5][6]. The identification of cancer cells, the finding of the most effective antibodies for neutralizing the deadly Ebola virus, and determining which antibodies fight various diseases and viruses [7][8], in addition to the alterations of metabolic pathways determined for different pathologic status, exemplify some applications of the importance of chromatography in the advancement of medical science supported in the early diagnosis of certain pathologies [9], and in the evolution of therapeutic efficacy [10], with personal, social, and economic impact.

In the food science and food industry, chromatographic techniques are used for the establishment of the “foodprint” through the identification and characterization of different food components, including the volatile compounds, some of them associated with the aromatic and organoleptic characteristics of the food [11], vitamins [12], proteins

[13], amino acids [14], mono-(MUFAs) and polyunsaturated fatty acids (PUFAs) [15], carbohydrates, polysaccharides [16], and contaminants [17] together with their metabolism, toxicology, and food fate. Additionally, chromatographic techniques have proved to be powerful tools in defining the authenticity and typicality of foods [18], helping to protect them from potential frauds and/or adulterations. In this context, an example can be referred to, the horsemeat scandal in 2013, which positioned chromatography (LC–MS) as the frontrunner in the analysis of processed meat composition in contrast to the ineffectiveness of traditional food analysis methods [19].

Chromatographic techniques also played an important role in the advancements verified in agricultural sciences. The identification and monitoring of biotic and abiotic stress markers [20], and the definition of the state of maturation of certain products [21], help producers define harvest dates according to the intended maturation indicators and considering the organoleptic characteristics of the final product.

In environmental sciences, chromatographic systems also significantly contributed to the search for a cleaner and more sustainable environment. Many environmental pollutants used in agriculture including DDT, lindane, endrin, dieldrin, heptachlor, and chlordane [22], have long biological half-lives and tend to bioaccumulate, therefore, representing a serious threat to different forms of life in our planet, including ours. The identification of such agri-toxics was made thanks to chromatography (GC–ECD, GC–MS and LC–MS, depending on the target analytes). The progressive increase in the sensitivity and precision of chromatographic techniques facilitated the action of governmental agencies, industrial organizations, and research groups on the implementation of inspection guidelines and screening programs that are simultaneously beneficial to air, soil, water, and wildlife as well as to humanity.

In the chemical industry, air monitoring is used to identify and analyse different chemical compounds [23]. Chromatography also plays an important role in the synthesis of radiolabeled chemicals, such as ^{14}C-labeled compounds used as radiotracers in metabolism investigations [24].

In pharmacology and biotechnology, chromatography helped in several innovations and advances. From quality control to drug development, and from pharmacokinetics to pharmacodynamics [25], the pharmaceutical industry is heavily regulated to ensure the safety and efficacy of pharmaceutical products and meet the specific guidelines implemented by regulatory authorities worldwide. Additionally, the OMICs platforms allow us to increase the knowledge of the human body at the cellular level since the metabolome offers a powerful way to determine and evaluate the cell response to external and internal stimuli. This understanding is also important to study the impact of drugs and therapeutic procedures at the cellular level. **Figure 2** shows a network revealing the interaction and importance of chromatography in the most diverse fields of science, knowledge, and technology, evidencing its impact on those areas.

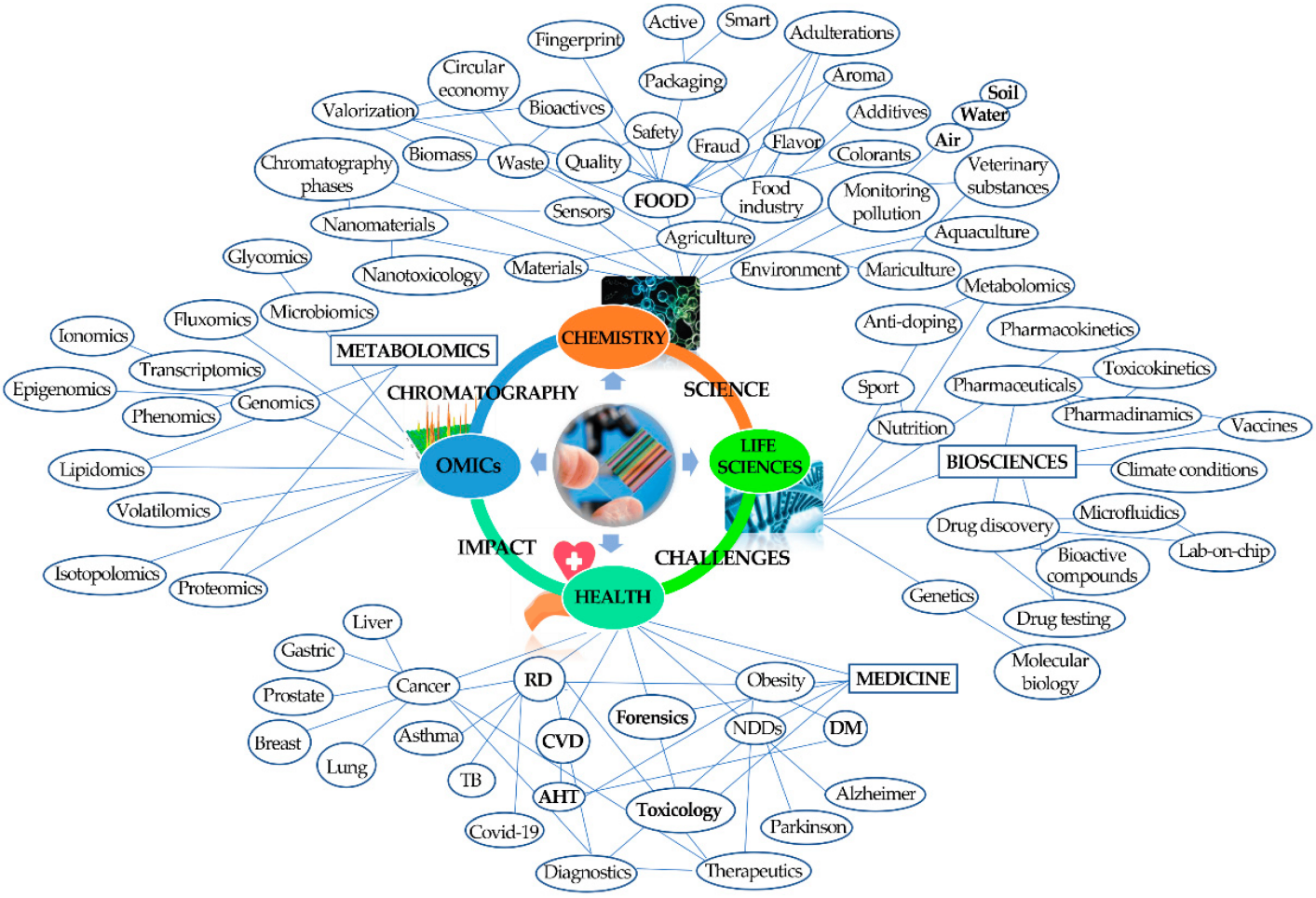

Figure 2. Co-occurrence network map related to the importance of chromatography in some fields of science.

The growing importance of chromatographic systems in hospitals, research laboratories, and industries expressed, for instance, by the high number of publications (approximately 90,000 papers published in chromatography in the last three and a half years), reveals the enormous contribution of the technique to the advancement of science in various branches of knowledge. Furthermore, its impact in helping the societal and technological challenges posed mainly by climate changes and the COVID-19 pandemic, is outstanding. This contribution was also boosted by the development of other branches of separation science and analytical techniques, namely, MS and highly sensitive detection systems, which developed concomitantly. Overall, the progression of R&D and improvements in chromatographic techniques will continuously improve the accuracy and precision of analytical results, favoring growth and innovation opportunities. The aforementioned fields, among others, boosted several innovations and advancements in the chromatography equipment to improve the technique's sensitivity and resolution, allowing a wide range of coverage in terms of chemical nature and concentration of the target analytes. Additionally, the developments in column stationary phases favored the improvement of resolution power and increased the number of detectable metabolites. This is exemplified by the huge power of multidimensional chromatography techniques, such as two-dimensional gas chromatography (2D-GC commonly known as GC × GC) [26], and two-dimensional liquid chromatography (2D-LC) [27], for separating complex mixtures.

The scientific world has demanded better performance from chromatographic methods, both in terms of throughput and resolution. On the other hand, the high-resolution power and ultra-high performance, in terms of sensitivity, detection, and identification abilities served as a platform for the evolution of science in a wide range of areas.

2. Chromatography Helping in Societal Challenges

Herein, a set of scientific articles published in the last decade was selected to show how the data obtained from the chromatographic analysis can be essential to respond to current societal challenges, with a huge impact on our health and well-being, as well as on the valorization of natural resources and sustainability. Specific case studies are presented to highlight the importance of selecting appropriate chromatographic methods and equipment for the analyses of different types of analytes.

2.1. Chromatography-Based Metabolomics Utility to Unveil Disease and/or Physiological Status

The study of a disease, as well as its diagnostics, treatments (including drug development), and/or biomarkers is a very challenging subject matter, and their decoding may be outpaced using chromatographic tools. Indeed, health-related studies based on the human metabolome are quite challenging. The human metabolome is very complex, and the use of animal models may be required to help in a wide range of scientific queries, from basic science to the development of new therapies and vaccines, among others. Indeed, the human metabolome contains a vast chemical diversity of the low-molecular-weight substances that can be produced in cells along a metabolic process, which include amino acids, carbohydrates, lipids, nucleic acids, vitamins, organic acids, and volatile metabolites, among others. Moreover, each body fluid or cell type may present a distinctive metabolite profile (also considering a wide concentration range), which can depend on genetic and environmental factors [28]. As there is no analytical technique that can cover all the enumerated specificities, the combination of several analytical techniques may be crucial to have an in-depth metabolome characterization. One example was shown in the multi-omics approach that was performed to clarify the molecular connection between obesity development and the high-fat diet, using the mouse model C57BL/6J [29]. Herein, data RNA transcriptomics of tissues, the metabolomics of several body fluids (plasma and urine) and tissues (liver, adipose, and muscle), and metagenomics analysis were combined to achieve the goal. Hence, the mice metabolome was studied using diverse analytical techniques, namely: NMR (Nuclear Magnetic Resonance), which allows the analysis of non-polar and polar analytes, is highly reproducible, but has lower sensitivity; LC–MS has higher sensitivity and depending on the LC type can detect a broad range of components, and herein, a UPLC-qToFMS and UPLC-Q extractive high-resolution accurate mass (HRAM) were used, and the instrumental conditions were adjusted according to the sample type (e.g., different chromatographic columns—SeQuant ZIC-cHILIC, BEH C18, and HSS T3 (with <3 µm particle size), different mobile phases and so on); and GC–MS, which is also a very sensitive and reproducible technique, and herein, analytes were derivatized by silylation and analyzed by a GC-ToFMS, equipped with an RTX 5 column. The MS analyzers used here, namely, qToFMS and HRAM, are highly selective and sensitive, being extremely important for accurate metabolite identification and quantitation. The metabolites profile obtained from mice tissues and body fluids were combined and analyzed by a multi-block PCA (Principal Component Analysis) approach (**Figure 3**), which showed a

distinguished pattern regarding the type of diet: low-fat (LFD) and high fat (HFD). LC–MS urine profile was the most distinctive profile among these samples, and there were also alterations observed in the liver tricarboxylic acid (TCA) cycle intermediates concentrations (e.g., malate, fumarate, oxaloacetate), which corroborated the gene expression data obtained of the enzymes related to TCA cycle [29].

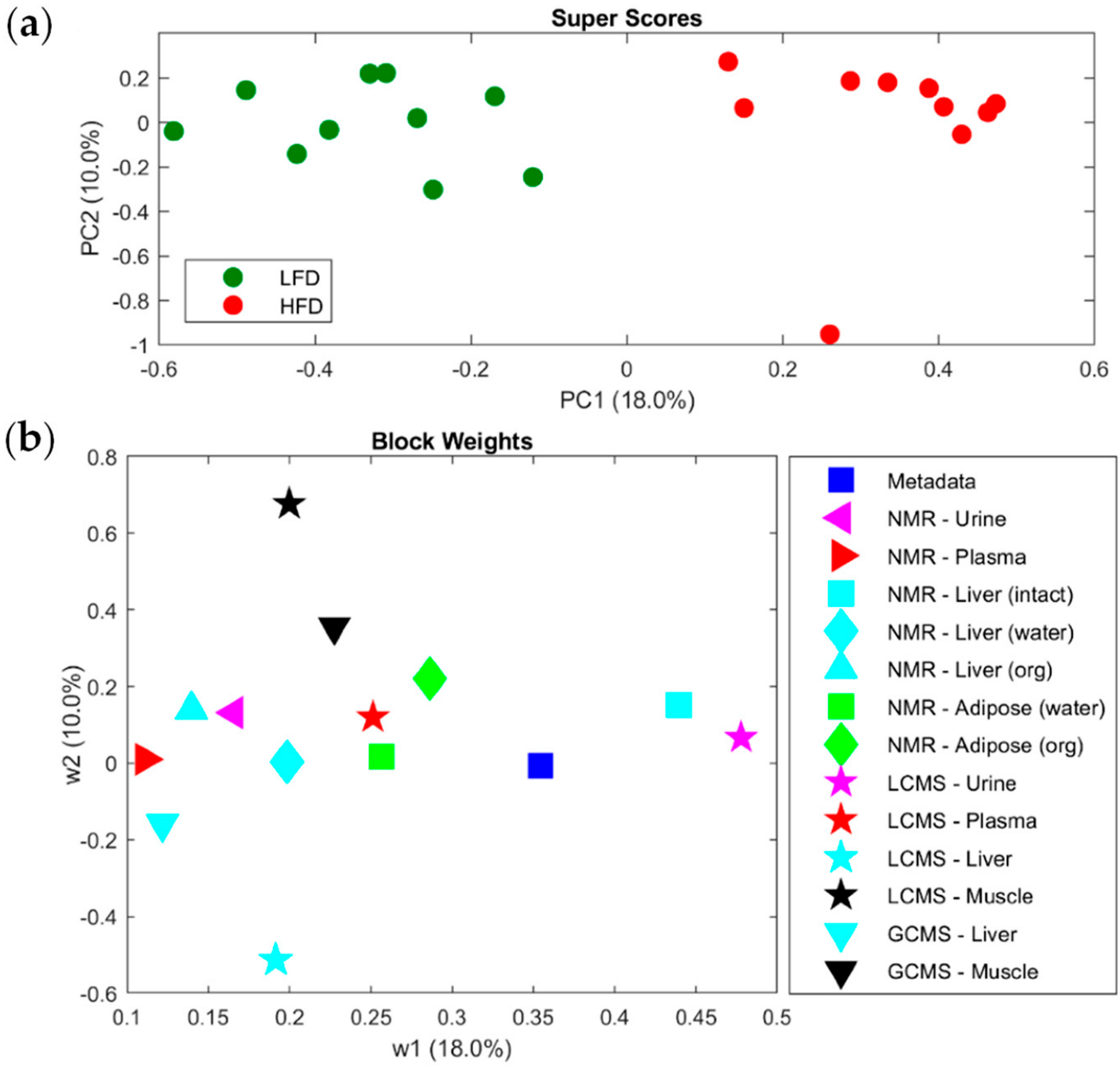

Figure 3. Multi-block PCA super scores plot (**a**) and block weights (**b**) of the body fluids and tissues, considering the metabolomics data obtained from mice fed with a low-fat diet (LFD) and high-fat diet (HFD) [29].

Reliable interpretations and, therefore, biological data mining of the huge amount of data that can be acquired by the chromatographic techniques, particularly the multidimensional ones, are vital, especially in health-related studies. In fact, omics data can be processed using several free available bioinformatics tools that integrate and

correlate the data with several databases [30], as previously shown with multi-block PCA. However, for deeper knowledge, omics data can be fused and integrated using artificial intelligence and machine learning approaches, which combine and process chromatographic data to help researchers and physicians to deepen understanding and create knowledge that supports their decisions [31][32][33]. This remarkable amount of data that is continuously been produced every day has been contributing to the construction of online databases, which systematize and may integrate several data domains, depending on the database [32].

One innovative and disruptive example that combines chromatography and artificial intelligence and machine learning approaches, in a clinical context, was a fast GC methodology that was developed for a possible non-invasive diagnosis of Parkinson's Disease (PD) using the odor profile of humans' skin sebum [34]. The developed artificial intelligence olfactory system consisted of three modules: an injection and preconcentration module (adsorbent tube filled with Tenax TA), a GC module (DB-1 column—1m), and a surface acoustic wave sensor detection module (Rayleigh wave gas sensor with a −69,766 Hz/ng sensitivity to mass deposition), with embedded machine learning (ML) algorithms. The linear detection range of the methodology varied from 0.025 to 50 mM. Three volatile organic compounds (VOCs) showed a significantly different profile between the PD patients and healthy controls, for instance, octanal, hexyl acetate, and perillic aldehyde. Depending on the ML algorithm, the models could achieve up to 92% regarding specificity and sensitivity. An accuracy of 70.8% was achieved for the classification model that was based on the significant features. This newly developed system has several advantages, namely, it is non-invasive, user friendly, convenient, and fast, which allows it to be easily implemented in hospitals and/or clinics to diagnose and monitor PD treatments. Still, several limitations need to be overcome, namely, co-elutions may occur, which may compromise the results; the diagnostic accuracy is still dependent on the training set dimension and population representativeness of the samples, which promotes a limited model utility for now; additionally, the biological relevance of hexyl acetate and perillic aldehyde is still not well-understood, which may require the addition of new biomarkers [34].

The complexity of the body fluids and tissue matrices, or even the detection of trace components require the use of highly sensitive and throughput methodologies, such as multidimensional chromatography. Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC–ToFMS) is a well-suited technique to perform personalized studies focused on individuals, capable to detect analytes at pg level, as it can be observed on the study of the usage impact of surgical face masks on young researchers (individual protection that became daily habit due to the COVID-19 disease), taking into consideration a normal working day [35]. For this study, exhaled breath condensate was the selected body fluid for the analysis, and its volatiles were extracted by SPME. A non-polar stationary phase (HP-5, 30 m) was used as the first column, which was combined with a polar stationary phase (DB-FFAP, 0.79 m) that was used as the second column, with these two columns being connected in series by a cryomodulator (highly recommended for the analysis of highly volatile metabolites). The high acquisition speed (ca. hundred spectra per second) makes ToFMS the most suitable analyzer for GC × GC once it provides enough data density, thus, allowing the accurate spectra deconvolution of overlapping peaks. The lipid peroxidation volatile profile achieved by GC × GC–ToFMS did not show significant changes along a working day, namely, aliphatic alkanes and aldehydes (which were previously associated with the lipid peroxidation process [36]). Additionally, heart rate and blood oxygen saturation count did not display significant changes, and the reported

values were within the conventional values predictable for a healthy adult. Thus, in the tested conditions, the use of face masks did not promote significant alterations in pulmonary hemodynamics, which might lead to hypoxia and resultant lipid peroxidation [35].

2.2. The Role of Chromatography to Respond Current Agri-Food Industries Challenges and Sustainability

Food consumption trends have been changing for years, and currently, consumers are looking for healthier products, not only in nutritional terms, but also considering food safety standards and the presence of bioactive components with beneficial properties (e.g., functional foods, nutraceuticals and so on) that are convenient, natural, and sustainable. Consumers are looking for local and organic products [37][38] presenting a lower ecological footprint, and fewer agrochemical treatments during their production, among others. To meet these requirements, food producers need to continuously develop new food products, and decisive and accurate analytical techniques are required to monitor food authentication and traceability, both ensuring food quality [39]. Moreover, the exaggerated competition in the production of certain food products has been promoting food fraud/adulteration, and chromatographic techniques play an important role in their monitoring. Another emerging topic is the amount of food loss and waste and how it should be reused. Indeed, ca. 90 million tons of food are wasted by the European Union each year [40], leading to significant environmental and economic issues, and food industries are one of the players involved. Therefore, there is an active search, for instance, using chromatographic techniques for new bioactive components and/or new functional food ingredients using agri-food industrial wastes or by-products in a wide range of applications, as it is already reported in several recent reviews [41][42][43][44].

Food matrices are complex due to the wide range of chemical components that they contain (e.g., different polarities, structures, concentration levels—from ng/kg to g/kg), which chemical analysis implies a selection of the proper analytical technique may fulfil the specific requirements of its analysis (target or untargeted analysis). For instance, GC-based methodologies showed to be a very valuable analytical technique to monitor food VOCs that may have an impact on their aroma if above their odor threshold [45]. Indeed, VOCs have been studied to allow the food's distinction regarding its geographical region, species or varieties, using mainly HS-SPME as the extraction technique, as shown in the following examples: apple (fruit and juice) and cider [46], broa (Portuguese maize bread) [47], grape [48][49], onion [50], saffron [11], olive oil [51], salt [52], rice [53][54], truffles [55], pears [56], among others. Considering the detection technique, gas chromatography-quadrupole mass spectrometry (GC-qMS), is the most used [11][46][48][50] that can be easily implemented in an industrial context. GC × GC–ToFMS, however, has also been used [47][49][52][56] due to lower detection limits, faster run times, and high resolution and peak capacity, promoting an in-depth food characterization. Nevertheless, GC × GC-based techniques have several limitations, such as high consumable costs, complex instrumentation, and the requirement of operational expertise. Since the type of analytes that are mainly present in these food matrices have polar groups in their chemical structure (e.g., acids, alcohols, aldehydes, esters, furans, ketones, lactones, norisoprenoids, pyrazines, terpenic compounds and so on), polar stationary phases are preferable for this analysis, namely, polyethylene glycol derivative phases (e.g., BP-20, SUPELCOWAX 10, and DB-FFAP) [11][46][48][50]. For GC × GC–ToFMS analysis, the combination of non-

polar/polar columns has been used, for instance (5%-phenyl)-methylpolysiloxane phase as the nonpolar columns (e.g., Equity-5, HP-5) and polyethylene glycol derivative phase (e.g., DB-FFAP) as the polar columns [47][49][52].

Enantioselective GC-based approaches are required for the analysis of chiral volatile components to assess the authenticity and quality of foods, and, for instance, the enantiomeric ratio has been used for the origin identification and adulteration of essential oils [57][58] and for the chiral contribution on baked/fermented teas [59][60], wines [61][62], among others. Particularly, in recent years, enantioselective multidimensional GC (enantio-MDGC) has been a key technique used for the separation of overlapping enantiomers, in a wide range of foods [63]. For instance, tea tree oil (TTO), extracted from *Melaleuca alternifolia*, is one of the most commercialized essential oils worldwide, whose demand is higher than its production, leading to intentional adulterations. The normative ISO 4730, which regulates the TTO chemical composition, has been continuously updated due to the outcomes achieved by the scientific research, which contributed to stricter requirements of the chromatographic profile at each update [64]. Enantioselective gas chromatography (eGC), particularly fast chiral methodologies based on GC–eGC-FID and eGC × GC-FID were applied to study the stereoisomeric ratios of limonene, terpinen-4-ol and terpineol in TTO [65]. The heart/cut approach (GC–eGC-FID) was achieved using a microfluidic Deans switch apparatus, with the Rxi-17 Sil MS used as the first-dimension column and Astec CHIRALDEX B-PM used as the second-dimension column; while the comprehensive approach (eGC × GC-FID) was realized with Astec CHIRALDEX B-PM as the first dimension column and SUPELCOWAX 10 as the second-dimension column. Both approaches used cryogenic modulators, ideal for volatile components. An example of the eGC × GC-FID contour plot of two TTO can be observed in **Figure 4**, in which it is possible to verify the adequate enantiomer separation of limonene, terpinen-4-ol, and terpineol without interferents [65]. The purposed chromatographic methodologies allowed a significant reduction in run time, from 75 min (ISO 4730, GC-FID) to 25 and less than 20 min for GC–eGC-FID and eGC × GC-FID, respectively [65]. Therefore, these fast and accurate approaches may act as trustworthy platforms for authenticity control of TTO [65], and they contributed to the amendment of ISO 4730:2017, which added the enantiomeric distribution of terpinen-4-ol that TTO must have in its composition, for instance: 67–71% (+, S) and 29–33% (−, R) [66].

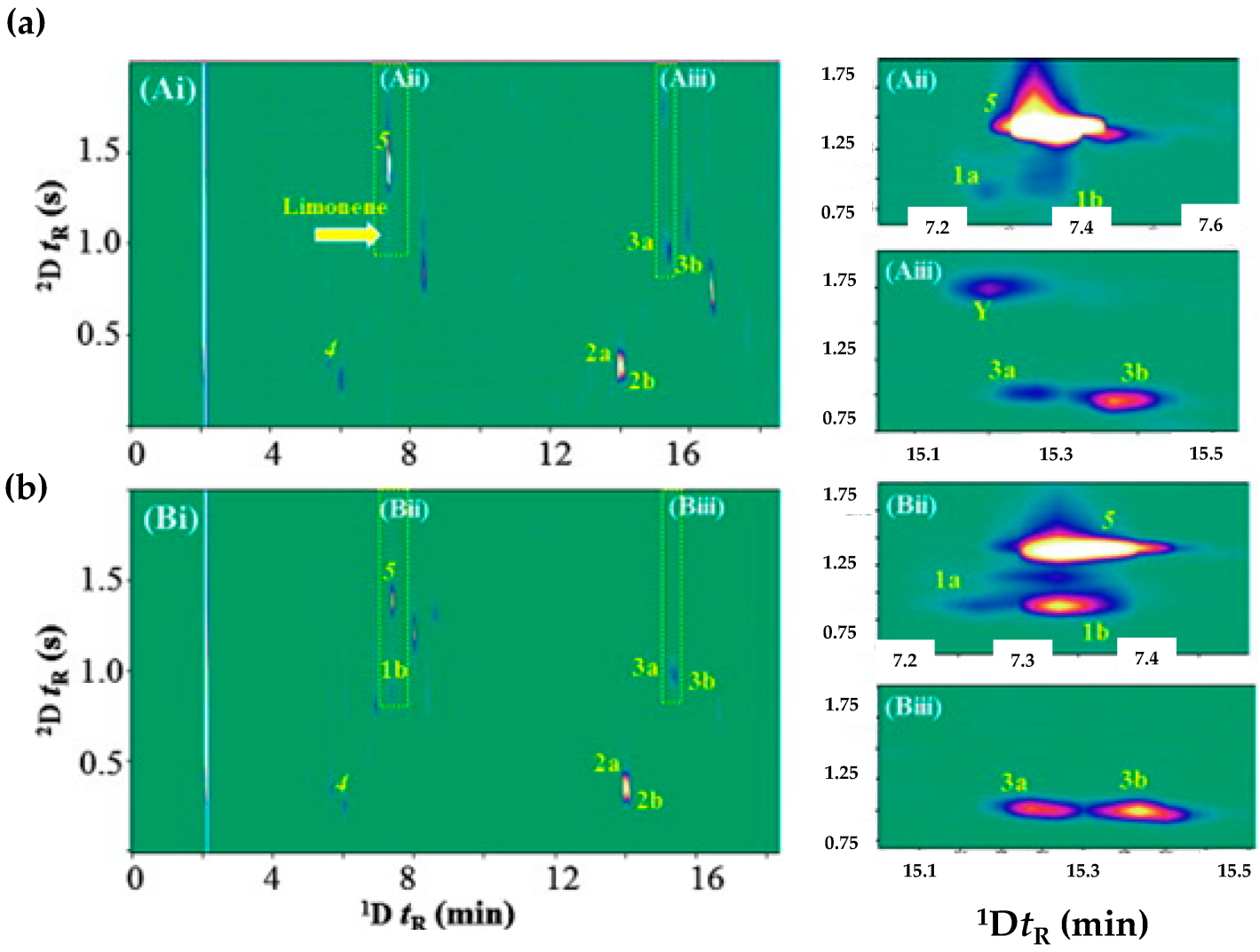

Figure 4. eGC × GC-FID contour plots of two TTO samples P1 (**a**) and C6 (**b**), and the expansion of rectangle region of limonene (Aii and Bii) and α-terpineol (Aiii and Biii) in TTO of samples P1 and C6, respectively. Components: 1(a), (−)-limonene; 1(b), (+)-limonene; 2(a), (+)-terpinen-4-ol; 2(b), (−)-terpinen-4-ol; 3(a), (−)-α-terpineol; 3(b), (+)-α-terpineol; 4, α-pinene; 5, p-cymene; Y, unknown compound. Reprinted from [65].

LC-HRMS can also be used to unveil the complexity of the food matrices and the great variability of the bioactive components, as this emerging technique guarantees the unambiguous determination of the elemental content of isobaric compounds and resolves the co-elution problems of isobaric compounds due to the type of analyzer, with ToF and Orbitrap the most frequently used [67]. One example is the use of a LC-QToFMS methodology to study saffron adulterations, which have been occurring with foreign plants that present similar color and morphology (the normative ISO 3632 evaluates and determines the saffron quality, of which detection limit of adulteration is up to 20%). Particularly, saffron adulteration has been performed with gardenia (*Gardenia jasminoides Ellis* of the *Rubiaceae* family), whose major glycoside is geniposide. For this, the better peak capacity and resolution of the LC-QToFMS methodology were achieved with the Ascentis Express Fused-core C18 column (fused-core particles composed of 2.7 µm particle size and 0.5 µm thick porous shell). This methodology was shown to be useful once it was able to simultaneously quantify glycosylated kaempferol derivatives, which were considered saffron

authenticity markers, and geniposide as an adulteration product in saffron [68]. Depending on the glycosylated kaempferol that was used, the minimum detectable adulteration of saffron varied between 0.2 and 2.5%, a fact that is significantly lower than the 20% of the ISO 3632.

The combination of several chromatographic techniques may be applied to a broader strategy for the multisensorial perception of foods due to the study of several dimensions associated with aroma perception, as was shown in the study of coffee odor and taste [69]. Indeed, there is a multimodal perception along with coffee or other food consumption, i.e., stimuli generate simultaneous (or closely simultaneous) data in more than one sensory modality. Therefore, the coffee multimodal perception was studied using the fingerprints of the volatile and non-volatile fractions and combined with sensory data [15]. The volatile profiles were obtained using HS-SPME combined with GC-qMS (equipped with a SolGelwax column). The non-volatile profiles were achieved using an LC-UV/DAD (equipped with a Platinum EPS C18 column), and analytes identification was confirmed using an LC–MS/MS with a triple quadrupole (equipped with an Ascentis Express C18 column). The three data domains were processed using unsupervised (PCA and MFA—multifactor authentication) and supervised (PLS—Partial Least Squares Regression) chemometrics tools. The developed regression models showed a key role of the volatiles in the sensorial characterization of the samples, with it being hypothesized that VOCs profile may be satisfactorily representative to define coffee flavor. Nevertheless, an integrated approach should be well-suited as a complement to sensory analysis, giving particular interest in the designing of new coffees (e.g., with different flavor profile). The main limitation of this approach is the consistency of the sensory data due to the subjects' subjectivity. These sensory prediction instrumental tools will require the acquisition of a significant amount of chemical and sensorial data in order to be more robust and reliable, and the use of artificial intelligence and machine learning algorithms will become useful tools to combine data for the understanding of such complex phenomenon as it is the multimodal flavor perception [69].

The in-depth chemical characterization of food wastes and by-products, as potential sources of high-added value compounds, may presuppose the design of an analytical strategy that includes a set of equipment according to the nature and concentration of the target analytes. For instance, the lipophilic components can be derivatized by silylation and analyzed by GC-MS [70][71][72][73], and the phenolic components can be analyzed using LC–MS [70][74][75]. Indeed, the integration of several analytical methodologies allows for a more comprehensive and detailed chemical characterization as was shown in the study of the potential bioactive components from *Passiflora mollissima* seeds [70]. A pressurized-liquid extraction was optimized to be applied to the seeds, in which the non-polar extract was derivatized by silylation and analyzed by GC-QToFMS (equipped with a DB-5 column); while the polar extract extracted from the defatted residue was analyzed by UHPLC-qToFMS/MS (equipped with a Zorbax Eclipse Plus C18 column), as observed in the workflow presented in **Figure 5**.

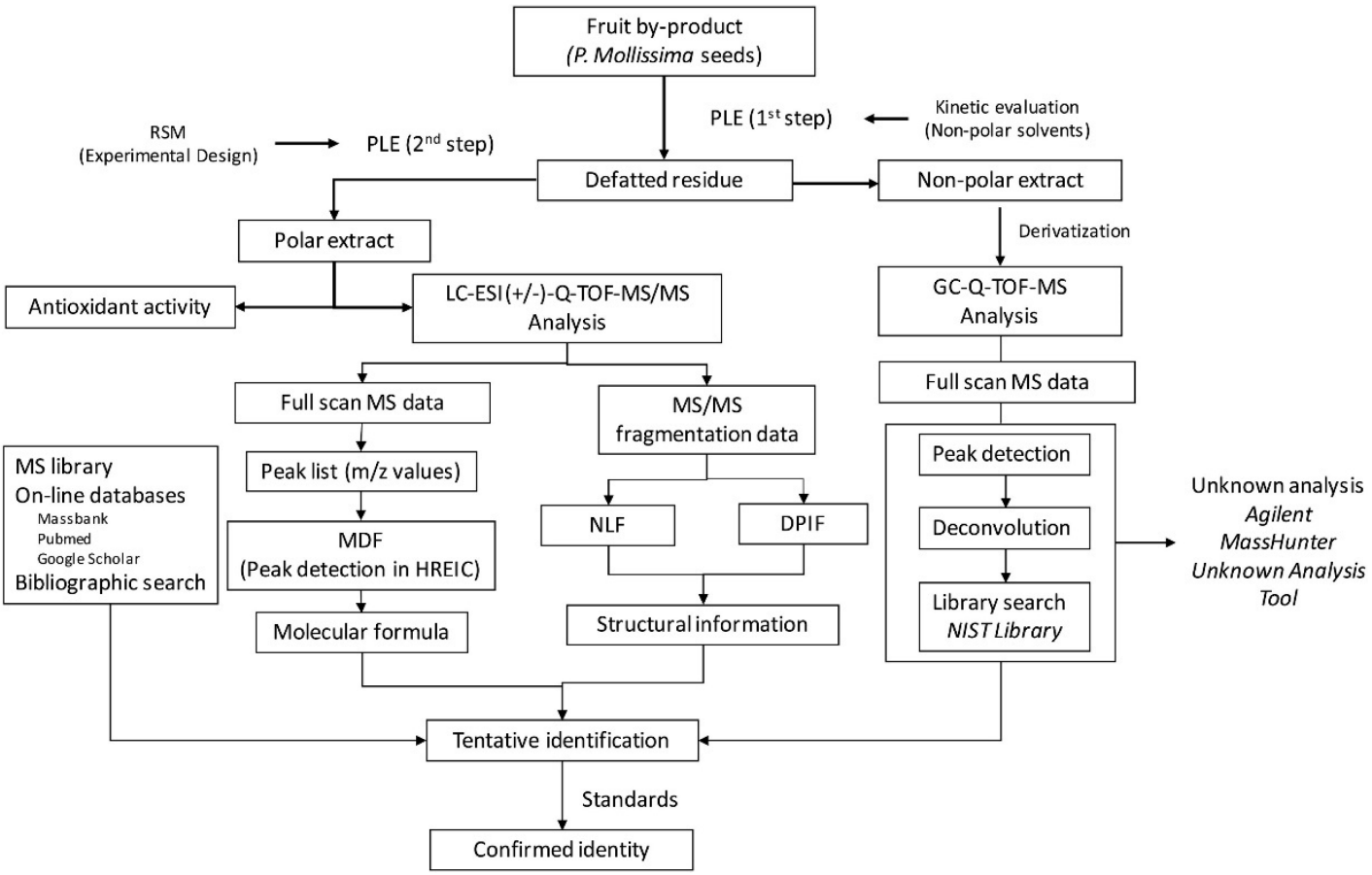

Figure 5. Workflow of the detailed chemical characterization of the bioactive components from *Passiflora mollissima* seeds. Legend: MDF: mass defect filtering; DPIF: diagnostic product ions filtering; HREIC: high-resolution extracted ion chromatograms; NLF: neutral loss filtering; PLE: pressurized liquid extraction; RSM: response surface methodology. Reprinted from [70].

The semi-targeted GC–QToFMS and untargeted UHPLC–qToFMS/MS strategies were successfully implemented to unravel the composition of *P. mollisima* seeds, particularly the polyphenols-rich extract, which was composed of flavonoids, flavanols, and proanthocyanidins, while the oily fraction was composed by MUFAs and PUFAs [70]. This strategy is particularly useful for the bioprospection of agri-food wastes and by-products, as it is extremely important to have high-resolution MS and MS in tandem (as it was used in the previous study, namely, QToFMS and qToFMS/MS) to perform putative identification of analytes, as their accurate identification is only proven through the authentic standards co-injection, and most of them are not commercially available or can be economically expensive. Nevertheless, these types of equipment require operational expertise and are quite expensive, which makes them still far from part of routine laboratory analysis, with quite a margin for improvement. Therefore, equipment such as HPLC-DAD/FLD can be used as a cost-effective option, particularly when authentic standards are available and there is previous knowledge regarding sample composition [76]. The use of libraries, as well as databases, will help in the putative identification of the new detected components. The comprehensive and detailed characterization of such complex natural matrices is crucial for the further steps of investigation, namely, regarding the bioprospection and consequent valorization of the food wastes and by-products as the potential source of bioactive compounds, that may be further used as food or cosmetic components.

2.3. Using Chromatography to Monitor Environmental Contaminants

The ecosystems are unfortunately widely contaminated with several ranges of chemicals, which according to European legislation, are defined as “substances (i.e., chemical elements and compounds) or groups of substances that are toxic, persistent and liable to bio-accumulate and other substances or groups of substances which give rise to an equivalent level of concern” [77]. Within these substances, included is microplastics and emergent pollutants (such as sucralose and other artificial sweeteners; nanomaterials; per- and polyfluoroalkyl substances; pharmaceutical and hormones; drinking water and swimming pool disinfection byproducts; sunscreens/UV filters; brominated and emerging flame retardants; dioxane; naphthenic acids; benzotriazoles and benzothiazoles; algal toxins; ionic liquids; halogenated methanesulfonic acid). Therefore, it is important to establish mitigation strategies to monitor and reduce these contaminations that may be present at trace levels (from parts-per-trillion, ppt, ng/L, to parts-per-billion, ppb, µg/L), and chromatographic techniques may be helpful in achieving this intention.

Microplastics (MP) contamination is not only restricted to marine ecosystems as it also affects the human food system, and their identification and quantification can be performed through visual recognition, spectroscopic analysis (e.g., FTIR), tagging methods, and chromatographic techniques. Indeed, sequential pyrolysis GC-MSD (mass selective detector), equipped with an HP-5 column, showed to be an appropriate tool for the identification of types of polymer of MP particles and associated organic plastic additives [78], particularly plasticizers (e.g., phthalates), flavoring agents (e.g., benzaldehyde), and antioxidants (e.g., 2,4-di-*tert*-butylphenol) in MP particles. The characteristic decomposition products allowed for the identification of isolated MP particles, comparing them with standards [78]. The main disadvantages of this method rely on the fact that only one particle can be analyzed per run, being non-appropriate for routine analysis; additionally, it is not adequate for the study of entire environmental samples, as a matrix effect occurs and it is very sensitive to contaminations. Another thermoanalytical method that combines thermogravimetric analysis coupled to solid-phase extraction (TGA–SPE) and thermal desorption GC–MSD (using an HP-1 MS column) was used to quantify polyethylene in several environmental samples (e.g., soil, suspended solids, or bivalves) [79]. This methodology showed to be robust, however, still requires further studies with a broader range of MP. The potential of chromatography has yet to be fully exploited, nevertheless, these thermoanalytical methods will allow the first sample screening to determine contamination levels, with posteriorly applied spectroscopic methods being used to obtain the precise determination of the number and size of the particles [80].

LC–MS/MS (with triple quadrupole) has been used to quantify MP, namely, polyethylene terephthalate (PET) and polycarbonate (PC) in the dust (important carrier of several contaminants), using alkali-assisted thermal depolymerization. The advantage of using LC–MS/MS methodology to quantify atmospheric MP is that there is no need for pretreatments of the samples, once it can be directly used, also a wide range of MP sizes can be analyzed [81][82][83]. For instance, the minimum limit of quantification (LOQ) of PET and PC, using LC–MS/MS, was 178.3 µg kg^{-1} and 27.7 µg kg^{-1}, respectively [81], the values of which were significantly lower than those using an LC-UV (1×10^6 µg kg^{-1} for PET) [84]. Despite the better sensitivity of LC–MS/MS, it is not routine laboratory equipment, requires qualified people to operate it, and is expensive.

Adsorptive microextraction techniques coupled to HPLC-DAD have been used to quantify contaminants in several types of water (e.g., seawater, wastewater, superficial water) from several sources, for instance: phenols that are intermediates in industrial processes (e.g., production of pesticides, explosives, pharmaceuticals, resins, among others) [85]; benzotriazoles (usually used as anti-corrosion additives, ultraviolet stabilizers, fungicides, or dyes), benzothiazoles (generally used as anti-corrosion additives, paper biocides, or herbicides and fungicides), and benzenesulfonamides (largely used as plasticizers, intermediate in sweeteners synthesis, or disinfectants) [86]; or anthropogenic drugs, such as caffeine and acetaminophen [87]. Despite the high selectivity of the adsorptive microextraction techniques and the requirement of low sample volume, the detection limits provided by HPLC-DAD may not be satisfactory. For instance, bisphenol-A (BPA) was quantified by BAμE-LD/HPLC-DAD (equipped with a Tracer excel 120 ODS-A column), with a detection limit of 0.3 μg L^{-1} [85], of which the value is significantly higher than reported concentrations of BPA in US waters (for instance 0.0009 μg/L) [88].

GC–MS/MS and LC–MS/MS were used to perform an ultra-trace analysis of 40 French bottled mineral glasses of water, in which a broad range of emergent contaminants was quantified, for instance, 118 pesticides and respective transformation products, 8 alkylphenols, 172 pharmaceuticals, 11 phthalates, 11 hormones, and 10 perfluoroalkyl substances (PFAS) [89]. Considering the nature of the contaminants, several analytical producers were applied to these mineral waters, namely: offline SPE LC–MS/MS (pharmaceuticals, hormones, pesticides, PFAS, and alkylphenols), online SPE LC–MS/MS (pharmaceuticals and pesticides), Stir Bar Sorptive Extraction (SBSE) GC–MS/MS (pesticides) and online SPME GC–MS/MS (pesticides, alkylphenols, and phthalates). These previous approaches were optimized to provide the lowest and most reliable limit of quantification (LOQ), without matrix interferents. Most of the LOQ were below 10 ng/L, and almost all contaminants (99.7%) registered concentrations lower than LOQ [89]. These waters did not present hormones and pharmaceuticals in their composition, which indicates the protection of springs utilized for bottling from contamination. However, some samples presented pesticides and their metabolites, PFASs, and alkylphenols in their composition at low levels (two times lower than French regulation of pesticides), which suggests the possible use of herbicides near the aquifers (surface water) and distant atmospheric transport and deposition (e.g., by rainfall). The importance of chromatography was well underlain by this previous example: on the one side, the developed tool helps the water industry in the monitoring contaminants at ultra-trace concentrations, and on the other side, the trustworthy measurements avoid false positive/negative results.

The structural and molecular characterization of water-soluble organic components from air particles can be studied by different analytical methodologies, and the most pertinent are systematized in **Figure 6**, considering their resolution with the mass of the water-soluble organic matter (WSOM) analyzed [90]. In fact, WSOM plays a key role at environmental, climate, and public health levels, nevertheless, the knowledge regarding their sources, composition, formation, and transformation mechanisms is still scarce, being a current topic of research. Online aerosol mass spectrometry (AMS) is the preferred technique to analyze WSOM, nevertheless, it cannot overcome the high-resolution achieved in multidimensional offline techniques. Indeed, great enhancement in the WSOM constituents' selectivity and molecular discrimination has been reached with the utilization of HR-MS detectors and their hyphenation with multidimensional chromatographic equipment, or in combination with EEM–PARAFAC (fluorescence excitation-emission matrices–parallel factor analysis) methods. Moreover, 1D NMR (mainly liquid-

state) has also been used for the structural characterization of aerosol WSOM, and multidimensional (2D or higher) liquid-state NMR arises as a very promising technique for this. Even so, 2D NMR still faces several limitations, such as the handling difficulties regarding the processing of overlapping data, the lack of analytical knowledge in the utilization of spectral editing techniques of NMR, and the requirement of chemometric tools that can handle big data sets, such as those generated by multidimensional approaches [90].

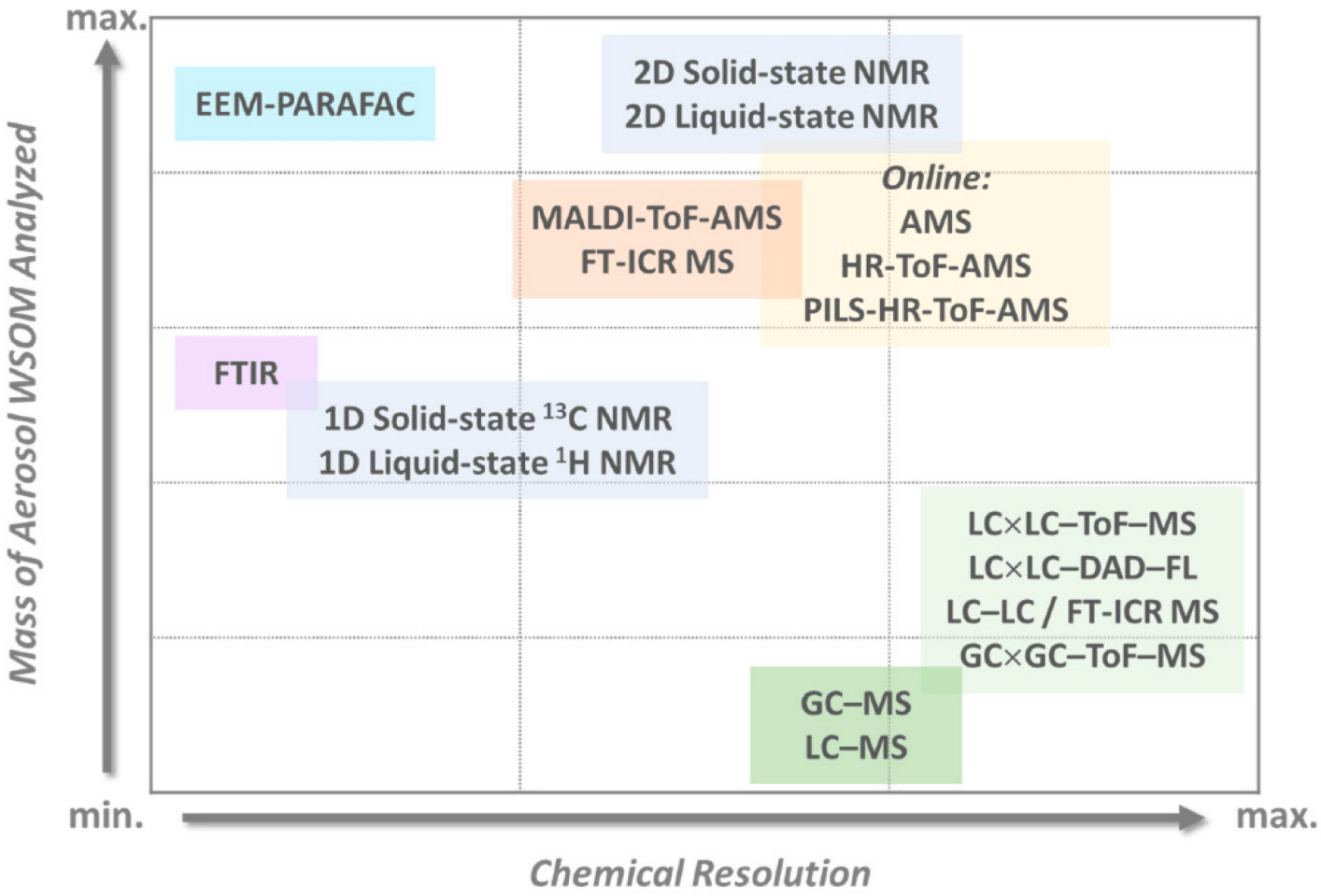

Figure 6. Schematic illustration of the analytical strategies most currently employed in the structural and chemical characterization of aerosol water soluble organic matter (WSOM), considering its amount versus the chemical resolution of the applied methodology [90].

References

1. Salter, T.L.; Magee, B.A.; Waite, J.H.; Sephton, M.A. Mass Spectrometric Fingerprints of Bacteria and Archaea for Life Detection on Icy Moons. Astrobiology 2022, 22, 143–157.
2. Leseigneur, G.; Bredehöft, J.H.; Gautier, T.; Giri, C.; Krüger, H.; MacDermott, A.J.; Meierhenrich, U.J.; Caro, G.M.M.; Raulin, F.; Steele, A.; et al. COSAC's Only Gas Chromatogram Taken on Comet 67P/Churyumov-Gerasimenko. ChemPlusChem 2022, 87, e202200116.

3. Cassaro, A.; Pacelli, C.; Baqué, M.; Cavalazzi, B.; Gasparotto, G.; Saladino, R.; Botta, L.; Böttger, U.; Rabbow, E.; de Vera, J.; et al. Investigation of fungal biomolecules after Low Earth Orbit exposure: A testbed for the next Moon missions. Environ. Microbiol. 2022, 24, 2938–2950.
4. Silva, C.L.; Perestrelo, R.; Capelinha, F.; Tomás, H.; Câmara, J.S. An integrative approach based on GC–qMS and NMR metabolomics data as a comprehensive strategy to search potential breast cancer biomarkers. Metabolomics 2021, 17, 72.
5. Lee, H.; Kang, S.J.; Lee, J.; Park, K.H.; Rhee, W.J. Isolation and Characterization of Urinary Extracellular Vesicles from Healthy Donors and Patients with Castration-Resistant Prostate Cancer. Int. J. Mol. Sci. 2022, 23, 7134.
6. Telleria, O.; Alboniga, O.E.; Clos-Garcia, M.; Nafría-Jimenez, B.; Cubiella, J.; Bujanda, L.; Falcón-Pérez, J.M. A Comprehensive Metabolomics Analysis of Fecal Samples from Advanced Adenoma and Colorectal Cancer Patients. Metabolites 2022, 12, 550.
7. Avataneo, V.; De Nicolò, A.; Cusato, J.; Antonucci, M.; Manca, A.; Palermiti, A.; Waitt, C.; Walimbwa, S.; Lamorde, M.; Di Perri, G.; et al. Development and validation of a UHPLC-MS/MS method for quantification of the prodrug remdesivir and its metabolite GS-441524: A tool for clinical pharmacokinetics of SARS-CoV-2/COVID-19 and Ebola virus disease. J. Antimicrob. Chemother. 2020, 75, 1772–1777.
8. Viode, A.; Smolen, K.K.; Fatou, B.; Wurie, Z.; Van Zalm, P.; Konde, M.K.; Keita, B.M.; Ablam, R.A.; Fish, E.N.; Steen, H. Plasma Proteomic Analysis Distinguishes Severity Outcomes of Human Ebola Virus Disease. mBio 2022, 13, e0056722.
9. Ahmed, N.; Kidane, B.; Wang, L.; Nugent, Z.; Moldovan, N.; McElrea, A.; Shariati-Ievari, S.; Qing, G.; Tan, L.; Buduhan, G.; et al. Metabolic Alterations in Sputum and Exhaled Breath Condensate of Early Stage Non-Small Cell Lung Cancer Patients After Surgical Resection: A Pilot Study. Front. Oncol. 2022, 12, 874964.
10. Bargaje, M.; Bharaswadkar, S.; Lohidasan, S.; Panda, B.K. Plasma drug concentrations of 4-drug fixed-dose combination regimen and its efficacy for treatment of pulmonary tuberculosis under National Tuberculosis Elimination Programme: A prospective pilot study. Indian J. Tuberc. 2021, 69, 311–319.
11. Cozzolino, R.; Stocchero, M.; Perestrelo, R.; Câmara, J.S. Comprehensive Evaluation of the Volatomic Fingerprint of Saffron from Campania towards Its Authenticity and Quality. Foods 2022, 11, 366.
12. Yang, J.; Wilson, I.; Rainville, P. Evaluation of hybrid surface technology for the analysis of the B-group vitamins by LC-ESI-MS/MS. J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci. 2022, 1204, 123336.

13. Liu, X.; Tian, X.; Qinghong, S.; Sun, H.; Jing, L.; Tang, X.; Guo, Z.; Liu, Y.; Wang, Y.; Ma, J.; et al. Characterization of LC-MS based urine metabolomics in healthy children and adults. PeerJ 2022, 10, e13545.
14. Muresan, A.A.; Rusu, A.; Roman, G.; Bala, C. Metabolomic Analysis of Normal Weight, Healthy and Unhealthy Obesity: Amino Acid Change Across the Spectrum of Metabolic Wellbeing in Women. Acta Endocrinol. 2021, 17, 427–431.
15. Kheiri, A.; Aliakbarlu, J.; Tahmasebi, R. Antioxidant potential and fatty acid profile of fish fillet: Effects of season and fish species. Vet. Res. Forum 2022, 13, 91–99.
16. Luo, B.; Lv, J.; Li, K.; Liao, P.; Chen, P. Structural Characterization and Anti-inflammatory Activity of a Galactorhamnan Polysaccharide from Citrus medica L. var. sarcodactylis. Front. Nutr. 2022, 9, 916976.
17. Dhanshetty, M.; Shinde, R.; Goon, A.; Oulkar, D.; Elliott, C.T.; Banerjee, K. Analysis of aflatoxins and ochratoxin a in chilli powder using ultrahigh performance liquid chromatography with fluorescence detection and tandem mass spectrometry. Mycotoxin Res. 2022, 38, 193–203.
18. Medina, S.; Perestrelo, R.; Silva, P.; Pereira, J.A.; Câmara, J.S. Current trends and recent advances on food authenticity technologies and chemometric approaches. Trends Food Sci. Technol. 2019, 85, 163–176.
19. Focker, M.; Borne, B.H.P.; Fischer, M.; Schuh, E.; Mader, A.; Andersson, M.G.; Ali, B.M.; Fels-Klerx, H.J. Interactions between risk assessors and risk managers during three major food incidents in Europe. J. Food Sci. 2021, 86, 3611–3627.
20. Ádám, A.L.; Kátay, G.; Künstler, A.; Király, L. Detection of Lipid Peroxidation-Derived Free Azelaic Acid, a Biotic Stress Marker and Other Dicarboxylic Acids in Tobacco by Reversed-Phase HPLC-MS Under Non-derivatized Conditions. Methods Mol. Biol. 2022, 2526, 191–200.
21. Niimi, J.; Deveau, A.; Splivallo, R. Geographical-based variations in white truffle Tuber magnatum aroma is explained by quantitative differences in key volatile compounds. New Phytol. 2021, 230, 1623–1638.
22. Omeje, J.S.; Asegbeloyin, J.N.; Ihedioha, J.N.; Ekere, N.R.; Ochonogor, A.E.; Abugu, H.O.; Alum, O.L. Monitoring of pesticide residues in fresh fruits and vegetables available in Nigerian markets and assessment of their associated health risks. Environ. Monit. Assess. 2022, 194, 516.
23. Rawat, S.; Singh, J. Synthesis of nZnO from waste batteries by hydrometallurgical method for photocatalytic degradation of organic pollutants under visible light irradiation. J. Environ. Manag. 2022, 318, 115518.
24. Yang, J.; Zhang, G.; Wang, Z.; Meng, J.; Wen, H. Metabolic Study of Stable Isotope Labeled Indolinone Derivative in Hepatocyte Cell by UPLC/Q TOF MS. J. Am. Soc. Mass Spectrom. 2021, 32, 1538–1544.

25. Zeng, W.; Lao, S.; Guo, Y.; Wu, Y.; Huang, M.; Tomlinson, B.; Zhong, G. The Influence of EGCG on the Pharmacokinetics and Pharmacodynamics of Bisoprolol and a New Method for Simultaneous Determination of EGCG and Bisoprolol in Rat Plasma. Front. Nutr. 2022, 9, 907986.
26. Amaral, M.S.S.; Nolvachai, Y.; Marriott, P.J. Comprehensive Two-Dimensional Gas Chromatography Advances in Technology and Applications: Biennial Update. Anal. Chem. 2020, 92, 85–104.
27. Stoll, D.R.; Carr, P.W. Two-Dimensional Liquid Chromatography: A State of the Art Tutorial. Anal. Chem. 2017, 89, 519–531.
28. Erjavec, G.N.; Konjevod, M.; Perkovic, M.N.; Strac, D.S.; Tudor, L.; Barbas, C.; Grune, T.; Zarkovic, N.; Pivac, N. Short overview on metabolomic approach and redox changes in psychiatric disorders. Redox Biol. 2018, 14, 178–186.
29. Sundekilde, U.K.; Yde, C.C.; Honore, A.H.; Rae, J.M.C.; Burns, F.R.; Mukerji, P.; Mawn, M.P.; Stenman, L.; Dragan, Y.; Glover, K.; et al. An Integrated Multi-Omics Analysis Defines Key Pathway Alterations in a Diet-Induced Obesity Mouse Model. Metabolites 2020, 10, 80.
30. Alonso, A.; Marsal, S.; Juliã, A. Analytical Methods in Untargeted Metabolomics: State of the Art in 2015. Front. Bioeng. Biotechnol. 2015, 3, 23.
31. Ahmed, Z. Practicing precision medicine with intelligently integrative clinical and multi-omics data analysis. Hum. Genom. 2020, 14, 35.
32. Branco, I.; Choupina, A. Bioinformatics: New tools and applications in life science and personalized medicine. Appl. Microbiol. Biotechnol. 2021, 105, 937–951.
33. Mattrey, F.T.; Makarov, A.A.; Regalado, E.L.; Bernardoni, F.; Figus, M.; Hicks, M.B.; Zheng, J.; Wang, L.; Schafer, W.; Antonucci, V.; et al. Current challenges and future prospects in chromatographic method development for pharmaceutical research. TrAC Trends Anal. Chem. 2017, 95, 36–46.
34. Fu, W.; Xu, L.; Yu, Q.; Fang, J.; Zhao, G.; Li, Y.; Pan, C.; Dong, H.; Wang, D.; Ren, H.; et al. Artificial Intelligent Olfactory System for the Diagnosis of Parkinson’s Disease. ACS Omega 2022, 7, 4001–4010.
35. Costa, C.P.; Marques, J.; Silva, D.; Barbosa, C.; Oliveira, A.S.; Santos, M.; Rocha, S.M. Metabolomics Profiling of Human Exhaled Breath Condensate by SPME/GC×GC-ToFMS: Exploratory study on the use of face masks at the level of lipid peroxidation volatile markers. Microchem. J. 2021, 171, 106830.
36. Caldeira, M.; Perestrelo, R.; Barros, A.; Bilelo, M.; Morête, A.; Câmara, J.; Rocha, S. Allergic asthma exhaled breath metabolome: A challenge for comprehensive two-dimensional gas chromatography. J. Chromatogr. A 2012, 1254, 87–97.

37. Bosona, T.; Gebresenbet, G. Swedish Consumers' Perception of Food Quality and Sustainability in Relation to Organic Food Production. Foods 2018, 7, 54.
38. Lauterbach, J.; Bantle, C. "For More Diversity, Better Taste and My Own Health" Exploring Organic Consumers' Purchasing Motives for Heirloom Vegetable Varieties. Sustainability 2022, 14, 4068.
39. Saadat, S.; Pandya, H.; Dey, A.; Rawtani, D. Food forensics: Techniques for authenticity determination of food products. Forensic Sci. Int. 2022, 333, 111243.
40. Stenmarck, Å.; Jensen, C.; Quested, T.; Moates, G.; Cseh, B.; Juul, S.; Parry, A.; Politano, A.; Redlingshofer, B.; Scherhaufer, S.; et al. Estimates of European Food Waste Levels—Fusions EU Project; IVL Swedish Environmental Research Institute: Stockholm, Sweden, 2014; pp. 1–80.
41. Difonzo, G.; de Gennaro, G.; Pasqualone, A.; Caponio, F. Potential use of plant-based by-products and waste to improve the quality of gluten-free foods. J. Sci. Food Agric. 2022, 102, 2199–2211.
42. Gil-Martín, E.; Forbes-Hernández, T.; Romero, A.; Cianciosi, D.; Giampieri, F.; Battino, M. Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. Food Chem. 2022, 378, 131918.
43. Patra, A.; Abdullah, S.; Pradhan, R.C. Review on the extraction of bioactive compounds and characterization of fruit industry by-products. Bioresour. Bioprocess. 2022, 9, 14.
44. Tapia-Quirós, P.; Montenegro-Landívar, M.F.; Reig, M.; Vecino, X.; Cortina, J.L.; Saurina, J.; Granados, M. Recovery of Polyphenols from Agri-Food By-Products: The Olive Oil and Winery Industries Cases. Foods 2022, 11, 362.
45. Rocha, S.M.; Costa, C.P.; Martins, C. Aroma Clouds of Foods: A Step Forward to Unveil Food Aroma Complexity Using GC × GC. Front. Chem. 2022, 10, 820749.
46. Medina, S.; Perestrelo, R.; Pereira, R.; Câmara, J.S. Evaluation of Volatilomic Fingerprint from Apple Fruits to Ciders: A Useful Tool to Find Putative Biomarkers for Each Apple Variety. Foods 2020, 9, 1830.
47. Bento-Silva, A.; Duarte, N.; Santos, M.; Costa, C.P.; Patto, M.C.V.; Rocha, S.M.; Bronze, M.R. Comprehensive Two-Dimensional Gas Chromatography as a Powerful Strategy for the Exploration of Broas Volatile Composition. Molecules 2022, 27, 2728.
48. Perestrelo, R.; Barros, A.S.; Rocha, S.M.; Câmara, J.S. Establishment of the varietal profile of Vitis vinifera L. grape varieties from different geographical regions based on HS-SPME/GC–qMS combined with chemometric tools. Microchem. J. 2014, 116, 107–117.
49. Petronilho, S.; Rudnitskaya, A.; Coimbra, M.; Rocha, S. Comprehensive Study of Variety Oenological Potential Using Statistic Tools for the Efficient Use of Non-Renewable Resources.

Appl. Sci. 2021, 11, 4003.

50. Fernandes, S.; Gois, A.; Mendes, F.; Perestrelo, R.; Medina, S.; Câmara, J.S. Typicality Assessment of Onions (Allium cepa) from Different Geographical Regions Based on the Volatile Signature and Chemometric Tools. Foods 2020, 9, 375.
51. Quintanilla-Casas, B.; Torres-Cobos, B.; Guardiola, F.; Servili, M.; Alonso-Salces, R.M.; Valli, E.; Bendini, A.; Toschi, T.G.; Vichi, S.; Tres, A. Geographical authentication of virgin olive oil by GC–MS sesquiterpene hydrocarbon fingerprint: Verifying EU and single country label-declaration. Food Chem. 2022, 378, 132104.
52. Silva, I.; Coimbra, M.A.; Barros, A.; Marriott, P.; Rocha, S.M. Can volatile organic compounds be markers of sea salt? Food Chem. 2015, 169, 102–113.
53. Asimi, S.; Ren, X.; Zhang, M.; Li, S.; Guan, L.; Wang, Z.; Liang, S.; Wang, Z. Fingerprinting of Volatile Organic Compounds for the Geographical Discrimination of Rice Samples from Northeast China. Foods 2022, 11, 1695.
54. Zhao, Q.; Xi, J.; Xu, D.; Jin, Y.; Wu, F.; Tong, Q.; Yin, Y.; Xu, X. A comparative HS-SPME/GC-MS-based metabolomics approach for discriminating selected japonica rice varieties from different regions of China in raw and cooked form. Food Chem. 2022, 385, 132701.
55. Kalogiouri, N.P.; Manousi, N.; Paraskevopoulou, A.; Mourtzinos, I.; Zachariadis, G.A.; Rosenberg, E. Headspace Solid-Phase Microextraction Followed by Gas Chromatography-Mass Spectrometry as a Powerful Analytical Tool for the Discrimination of Truffle Species According to Their Volatiles. Front. Nutr. 2022, 9, 856250.
56. Wang, C.; Zhang, W.; Li, H.; Mao, J.; Guo, C.; Ding, R.; Wang, Y.; Fang, L.; Chen, Z.; Yang, G. Analysis of Volatile Compounds in Pears by HS-SPME-GC×GC-TOFMS. Molecules 2019, 24, 1795.
57. Capetti, F.; Marengo, A.; Cagliero, C.; Liberto, E.; Bicchi, C.; Rubiolo, P.; Sgorbini, B. Adulteration of Essential Oils: A Multitask Issue for Quality Control. Three Case Studies: Lavandula angustifolia Mill., Citrus limon (L.) Osbeck and Melaleuca alternifolia (Maiden & Betche) Cheel. Molecules 2021, 26, 5610.
58. Ojha, P.K.; Poudel, D.K.; Dangol, S.; Rokaya, A.; Timsina, S.; Satyal, P.; Setzer, W.N. Volatile Constituent Analysis of Wintergreen Essential Oil and Comparison with Synthetic Methyl Salicylate for Authentication. Plants 2022, 11, 1090.
59. Ma, W.; Zhu, Y.; Shi, J.; Wang, J.; Wang, M.; Shao, C.; Yan, H.; Lin, Z.; Lv, H. Insight into the volatile profiles of four types of dark teas obtained from the same dark raw tea material. Food Chem. 2021, 346, 128906.
60. Zhu, Y.; Yan, H.; Zhang, Z.-F.; Zeng, J.-M.; Zhang, Y.; Wang, J.-T.; Ma, W.-J.; Wang, M.-Q.; Peng, Q.-H.; Lv, H.-P.; et al. Assessment of the contribution of chiral odorants to aroma property of

baked green teas using an efficient sequential stir bar sorptive extraction approach. Food Chem. 2021, 365, 130615.

61. Khvalbota, L.; Machyňáková, A.; Čuchorová, J.; Furdíková, K.; Špánik, I. Enantiomer composition of chiral compounds present in traditional Slovak Tokaj wines. J. Food Compos. Anal. 2020, 96, 103719.
62. Langen, J.; Wegmann-Herr, P.; Schmarr, H.-G. Quantitative determination of α-ionone, β-ionone, and β-damascenone and enantiodifferentiation of α-ionone in wine for authenticity control using multidimensional gas chromatography with tandem mass spectrometric detection. Anal. Bioanal. Chem. 2016, 408, 6483–6496.
63. Elbashir, A.A.; Aboul-Enein, H.Y. Multidimensional Gas Chromatography for Chiral Analysis. Crit. Rev. Anal. Chem. 2018, 48, 416–427.
64. Bejar, E. Adulteration of tea tree oil (Melaleuca alternifolia and M. linariifolia). Bot. Adulterants Bull. 2017, 1–8. Available online: https://www.melaleuca-alternifolia.com/kaufen/BAP-BABs-TTO-CC-v5.pdf (accessed on 28 July 2022).
65. Wong, Y.F.; West, R.N.; Chin, S.-T.; Marriott, P.J. Evaluation of fast enantioselective multidimensional gas chromatography methods for monoterpenic compounds: Authenticity control of Australian tea tree oil. J. Chromatogr. A 2015, 1406, 307–315.
66. ISO 4730:2017/Amd 1:2018; Essential oil of Melaleuca, Terpinen-4-Ol Type (Tea Tree Oil). ISO: Geneva, Switzerland, 2018.
67. Campmajó, G.; Núñez, N.; Núñez, O. The role of liquid chromatography-Mass spectrometry in food integrity and authenticity. In Mass Spectrometry—Future Perceptions and Applications Substances; Kamble, G.S., Ed.; IntechOpen: London, UK, 2019; pp. 3–20. ISBN 978-953-51-7845-3.
68. Guijarro-Díez, M.; Castro-Puyana, M.; Crego, A.L.; Marina, M.L. A novel method for the quality control of saffron through the simultaneous analysis of authenticity and adulteration markers by liquid chromatography-(quadrupole-time of flight)-mass spectrometry. Food Chem. 2017, 228, 403–410.
69. Bressanello, D.; Marengo, A.; Cordero, C.; Strocchi, G.; Rubiolo, P.; Pellegrino, G.; Ruosi, M.R.; Bicchi, C.; Liberto, E. Chromatographic Fingerprinting Strategy to Delineate Chemical Patterns Correlated to Coffee Odor and Taste Attributes. J. Agric. Food Chem. 2021, 69, 4550–4560.
70. Vivas, D.B.; Alvarez-Rivera, G.; Ibánez, E.; Parada-Alfonso, F.; Cifuentes, A. Integrated strategy for the extraction and profiling of bioactive metabolites from Passiflora mollissima seeds combining pressurized-liquid extraction and gas/liquid chromatography–high resolution mass spectrometry. J. Chromatogr. A 2019, 1595, 144–157.

71. Cuco, R.P.; Cardozo-Filho, L.; da Silva, C. Simultaneous extraction of seed oil and active compounds from peel of pumpkin (Cucurbita maxima) using pressurized carbon dioxide as solvent. J. Supercrit. Fluids 2019, 143, 8–15.
72. Patinha, S.; Murteira, J.V.; Costa, C.P.; Salvador, A.C.; Santos, S.A.O.; Silvestre, A.J.D.; Rocha, S.M. Elderberry Stalks as a Source of High-Value Phytochemical: Essential Minerals and Lipophilic Compounds. Appl. Sci. 2022, 12, 382.
73. Salvador, C.; Simões, M.M.Q.; Silva, A.M.S.; Santos, S.A.O.; Rocha, S.M.; Silvestre, A.J.D. Vine Waste Valorisation: Integrated Approach for the Prospection of Bioactive Lipophilic Phytochemicals. Int. J. Mol. Sci. 2019, 20, 4239.
74. Drosou, C.; Kyriakopoulou, K.; Bimpilas, A.; Tsimogiannis, D.; Krokida, M. A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification byproducts. Ind. Crops Prod. 2015, 75, 141–149.
75. Peralbo-Molina, Á.; Priego-Capote, F.; de Castro, M.D.L. Tentative Identification of Phenolic Compounds in Olive Pomace Extracts Using Liquid Chromatography–Tandem Mass Spectrometry with a Quadrupole–Quadrupole-Time-of-Flight Mass Detector. J. Agric. Food Chem. 2012, 60, 11542–11550.
76. Jurčević, I.L.; Dora, M.; Guberović, I.; Petras, M.; Brnčić, S.R.; Đikić, D.; Landeka, I.; Rimac, S. Wine Lees Polyphenols as a Novel Functional Bioactive Compound in the Protection against Oxidative Stress and Hyperlipidemia. Food Technol. Biotechnol. 2017, 55, 109–116.
77. European Comission. Our Oceans, Seas and Coasts, Descriptor D8 Documents: Contaminants. Available online: https://ec.europa.eu/environment/marine/good-environmental-status/descriptor-8/index_en.htm (accessed on 28 July 2022).
78. Fries, E.; Dekiff, J.H.; Willmeyer, J.; Nuelle, M.-T.; Ebert, M.; Remy, D. Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. Environ. Sci. Process. Impacts 2013, 15, 1949–1956.
79. Dümichen, E.; Barthel, A.-K.; Braun, U.; Bannick, C.G.; Brand, K.; Jekel, M.; Senz, R. Analysis of polyethylene microplastics in environmental samples, using a thermal decomposition method. Water Res. 2015, 85, 451–457.
80. Ivleva, N.P.; Wiesheu, A.C.; Niessner, R. Microplastic in Aquatic Ecosystems. Angew. Chem. Int. Ed. Engl. 2017, 56, 1720–1739.
81. Liu, C.; Li, J.; Zhang, Y.; Wang, L.; Deng, J.; Gao, Y.; Yu, L.; Zhang, J.; Sun, H. Widespread distribution of PET and PC microplastics in dust in urban China and their estimated human exposure. Environ. Int. 2019, 128, 116–124.
82. Luo, X.; Wang, Z.; Yang, L.; Gao, T.; Zhang, Y. A review of analytical methods and models used in atmospheric microplastic research. Sci. Total Environ. 2022, 828, 154487.

83. Zhang, J.; Wang, L.; Kannan, K. Microplastics in house dust from 12 countries and associated human exposure. Environ. Int. 2020, 134, 105314.
84. Tian, L.; Skoczynska, E.; Siddhanti, D.; van Putten, R.-J.; Leslie, H.A.; Gruter, G.-J.M. Quantification of polyethylene terephthalate microplastics and nanoplastics in sands, indoor dust and sludge using a simplified in-matrix depolymerization method. Mar. Pollut. Bull. 2022, 175, 113403.
85. Neng, N.R.; Nogueira, J.M.F. Determination of Phenol Compounds in Surface Water Matrices by Bar Adsorptive Microextraction-High Performance Liquid Chromatography-Diode Array Detection. Molecules 2014, 19, 9369–9379.
86. Ahmad, S.M.; Calado, B.B.; Oliveira, M.N.; Neng, N.R.; Nogueira, J. Bar Adsorptive Microextraction Coated with Carbon-Based Phase Mixtures for Performance-Enhancement to Monitor Selected Benzotriazoles, Benzothiazoles, and Benzenesulfonamides in Environmental Water Matrices. Molecules 2020, 25, 2133.
87. Silva, A.R.M.; Neng, N.R.; Nogueira, J.M.F. Multi-Spheres Adsorptive Microextraction (MSAμE)—Application of a Novel Analytical Approach for Monitoring Chemical Anthropogenic Markers in Environmental Water Matrices. Molecules 2019, 24, 931.
88. U.S. Environmental Protection Agency. Bisphenol A Action Plan (CASRN 80-05-7); U.S. Environmental Protection Agency: Washington, DC, USA, 2010; pp. 1–22.
89. Le Coadou, L.; Le Ménach, K.; Labadie, P.; Dévier, M.-H.; Pardon, P.; Augagneur, S.; Budzinski, H. Quality survey of natural mineral water and spring water sold in France: Monitoring of hormones, pharmaceuticals, pesticides, perfluoroalkyl substances, phthalates, and alkylphenols at the ultra-trace level. Sci. Total Environ. 2017, 603–604, 651–662.
90. Duarte, R.; Matos, J.; Duarte, A. Multidimensional Analytical Characterization of Water-Soluble Organic Aerosols: Challenges and New Perspectives. Appl. Sci. 2021, 11, 2539.