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Review

Omics-Integrated Approach (Metabolomics, Proteomics and Lipidomics) to Assess the Quality Control of Aquatic and Seafood Products

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Abstract: Since the demand for seafood products is growing and aquaculture provides more than fifty percent of the aquatic food as reported by FAO, the development of more accurate and sensitive analytical techniques in order to screen and evaluate the safety and quality of seafood products is needed. At this point, several omic techniques like proteomics, lipidomics, and metabolomics, or combinations of them, are used for integration into seafood processing and quality control. Moreover, according to the literature, using the respective techniques can prevent, control, and treat diseases in fish as well as address several issues in aquaculture. Proteomic techniques are used for the expression of proteins and their modifications. Metabolomic techniques are used for accurate identification of species, while lipidomics techniques are used for the identification of different or specific lipid molecules in fish species, as well as fatty acid composition and location distribution. This review is to cover the recent proteomics, metabolomics, and lipidomics studies on aquatic and seafood products in the areas of quality, safety, processing, and breeding of fish.

Keywords: metabolomics; proteomics; lipidomics; volatomics; metagenomics; seafood products; quality; safety; aquaculture; authentication

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1. Introduction

Aquatic and seafood products are one of the most important cardioprotective food groups with beneficial effects on human health because of the unsaturated fatty acids and primarily polyunsaturated fatty acids, such as docosahexaenoic acid, which are found in abundance. Moreover, higher aquatic and seafood product consumption was associated with lower risks of coronary heart diseases and improved brain development, visionary receptors, and the reproductive system [1]. Additionally, it has been stated that aquatic food products could rise by 21–44 million tons by 2050, and this is an increase of 36–74% evaluated against current yields [2]. As a result, it is critical for the maintenance of a safe and economically sustainable food supply chain, and it is also a prerequisite for the development of more sensitive, quicker, and reliable methods for quality and safety evaluation of aquatic food. This can be achieved by using omic technologies (metabolomics, proteomics, and lipidomics) jointly with classical microbiological methods and quality indicators like color, texture, and flavor [3].

Recently, the increasing attention on aquatic and seafood product-related themes, such as food quality and security, sensorial features, traceability, and conformity with regulatory necessities, has increased the development and application of analytical techniques and

statistical tools to recognize and anticipate these critical issues [4,5]. As an unindustrialized field in the biological sciences, metabolomics research emphases on small molecule metabolites of a particular system or organism at a precise time point [6,7].

By way of illustration, aquaculture involves severe criteria, comprising reproduction, temperature, microbial dynamics, water quality, feed, and environment; developments in omics technologies could offer new research methods. For example, Huete-Pérez and Quezada [8] stressed the genomics' role in exploring traits, sex determination, genetic construction, and environmental reactions. Qian et al. [9] studied transcriptomics, which aids in understanding the responses to stress vs. pathogens, osmotic pressure, and temperature variations. Metabolomics has already been effectively applied in examining aquatic and seafood products safety, quality, traceability, and authenticity. This tool was crucial to studying fish resource movement, ecological adaptation, biodiversity, measuring the impact of feed composition changes, and ensuring fish and seafood quality and safety. For instance, Alfaro and Young [10] highlighted metabolomics' implication in aquaculture, exploring its application in food and nutrition, production, post-harvest quality control, disease, and immunology. Because of its versatility, the employment of developed high-resolution mass spectrometry has incited the progressive application of omic technologies. For example, in lipidomics research, the Q-Orbitrap HRMS was successfully utilized to analyze the different parts of capelin to reveal fatty acids and lipid profiles [11], as well as the lipid profiling of the golden threadfin bream fish [12]. Furthermore, a lipidomics study and an analysis of the triglyceride levels of anchovy oils were also successfully carried out using LC–HRMS lipidomics combined with chemometrics [13]. On the other hand, proteomics could support reaching the higher aquatic and seafood product production scale with a maintainable quality. During different life phases of various fish species, proteomic changes were involved in significant events during development in a rapidly changing environment. In addition, the proteomics of fishes could aid in discovering changes taking place with regard to disease or extra environmental factors, viz. toxins, pollutants, and temperature fluctuations, that restrict normal metabolism in the fish body [14].

This review paper exemplifies the basics of different omics practices along with available bioinformatics methods for their applications in aquatic and seafood products, underlining the potential of these emerging approaches for ensuring their quality and safety.

Compared to most food products, aquatic and seafood products can be considered one of the most perishable products, with a short shelf life due to their biological origin, which is a mixture consisting mainly of proteins, lipids, and carbohydrates dispersed in water. For this reason, this review focuses mainly on these products and not on other types of food of animal origin, such as meat and meat products, milk and milk products, and eggs [15].

Research in the following databases took place, including Google Scholar, Scopus, and Science Direct, using the keywords metabolomics, lipidomics, proteomics, and seafood and quality.

2. Review Methodology

2.1. Inclusion Criteria and Relevant Screening of Studies

All original papers were assessed on the basis of (1) title, (2) abstract, and (3) full text screening. To remove outstandingly inappropriate articles, the screening title was the first stage. Regarding abstract screening, the abstract of articles, coordinated with consequent criteria, were selected. In addition, in order to prevent possible mistakes in language translation, available studies in English were involved. Similarly, only the data focusing on "metabolomics", "proteomics", "lipidomics", "transcriptomics", "aquatic and seafood products", "safety", and "control quality" were selected. Unpublished papers were not implicated, and the documents were collected for scientific articles only.

Concerning the screening of full text, here, full papers where the abstract was completed were examined, and unachievable manuscripts were eliminated. Only articles with accessible full texts were designated for data extraction/analysis. An article was selected if it satisfied the following criteria: (a) studies related to omic technologies for quality control of seafood and aquatic products; (b) detection of pathogens and spoilage microorganisms in aquatic food products; and (c) metabolomics, proteomics, lipidomics, and transcriptomics for metabolite detection in aquatic food products and traceability.

As described by the preceding meta-analysis investigations in food science and technology, and in order to limit any missteps in translation, only English language papers were incorporated. Papers not satisfying the set criteria were excluded. However, the prior condition led to a limited number of pertinent citations. Therefore, the manuscripts were included only if omic technologies and aquatic and seafood products were evaluated in the research paper. To attain references, the EndNote X7® software EndNote X7.2 Bld 8156 (Thomson Reuters, Toronto, ON, Canada) was employed.

2.2. Data Extraction

The data were carefully structured by the authors of this review. The collected data from each study were explained as study structures, corresponding to the first author, year of publication, omic technology, quality control, and safety. In order to search and recoup the research papers associated with these latter keywords, the selection was conducted, and the array of key collections in the online Scopus database was explored. Studies pertaining to conference abstracts, letters, patents, or review articles were excluded.

As shown in Figure 1, between 2014 and 2024, the number of publications augmented exponentially.

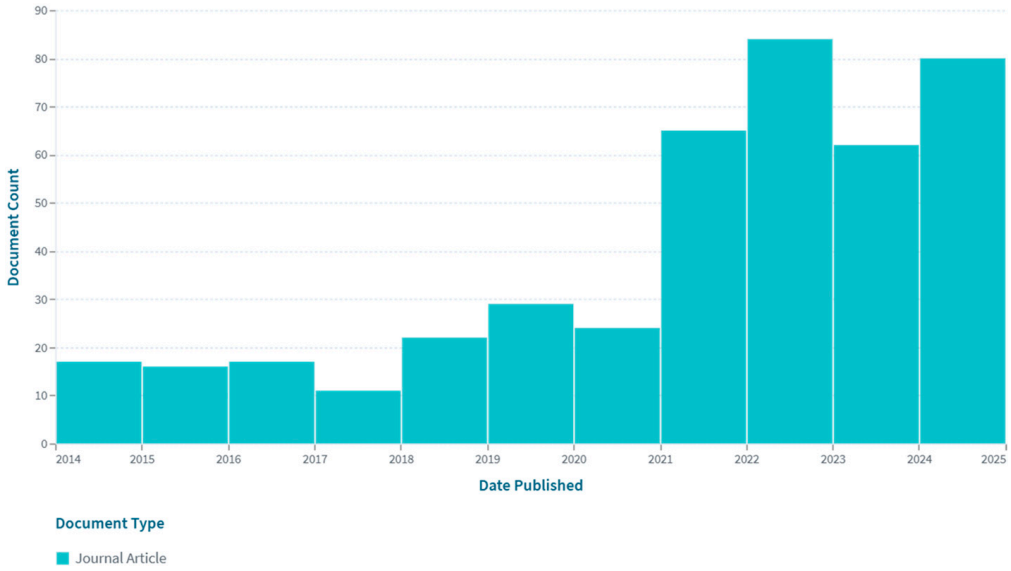

Figure 1. Number of publications on omic technology and aquatic food products between 2014 and 2024 (source: www.lens.org; accessed on 8 November 2024).

The research areas were evaluated according to the Journal Citation Report (JCR) assignments. The most allocated JCR subject categories were (i) biology, (ii) ecology, and (iii) chemistry. The omic technology and aquatic food products were linked with these fields (Figure 2).

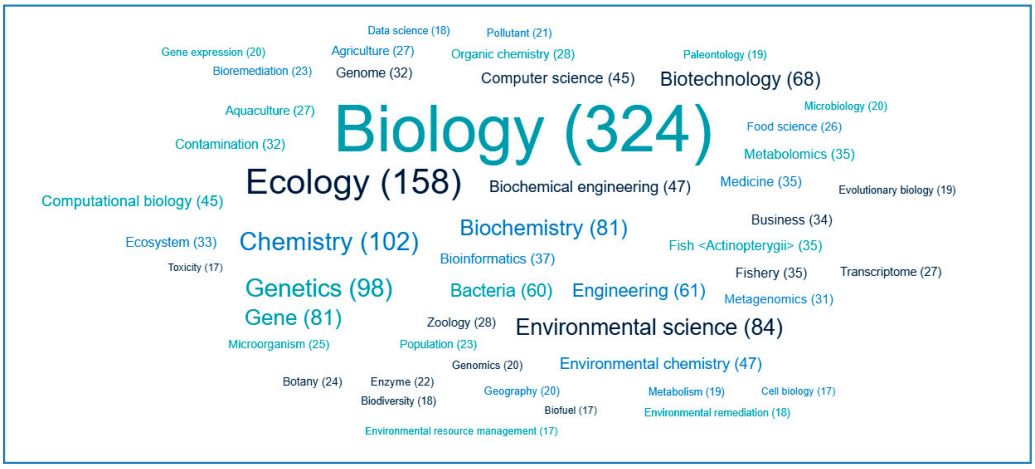

Figure 2. Publication subject linked to omic technology or aquatic food products (data obtained from the Lens website [www.lens.org]; accessed on 8 November 2024).

2.3. Short Overview of Omic Technologies

2.3.1. Principles of Omics Data

Metabolomics and Transcriptomics

Usually, metabolomics includes six stage keys: (1) experimental plan, (2) sample preparation, (3) investigation and data achievement, (4) raw data treatment, (5) statistical examination, and (6) interpretation [16]. Metabolomics utilized a diversity of analytical tools comprising MS (LC-MS and GC-MS), NMR, and vibrational-based approaches via Raman and IR spectroscopy. To comprehend the nutrient status in fish quality, NMR can be employed by computing the molecular relaxation time, accompanied by differentiating the food quality. GC-MS was applied to assess volatile molecules. Furthermore, amino acids, fatty acids, and other non-volatile compounds can be perceived via LC-MS. Compared to NMR, MS-based platforms are very perceptive, inexpensive, and deliver wide-ranging metabolites. Nonetheless, MS-based analyses are fewer reproducible, frequently need extra trial preparations, and are weaker in the acquisition of data when employing pre-separation plans.

In contrast to MS-based boards, an inclusive GC × GC-MS uses two consecutive pre-separation columns to enhance metabolite separation. This can decrease co-eluting metabolites in complicated biological trials, augment the sensibility, and considerably enlarge the range of perceptible metabolites [17]. DART-HR-MS (direct analysis in real-time high-resolution MS) is perfect for product authentication; its results are reproducible and sensitive, and it necessitates a minimal sample preparation [18]. The FT-ICR-MS (Fourier-transform ion cyclotron resonance MS) is a quantitative technique and provides an extensive range of metabolites with high resolution and sensitivity [19]. In addition, mutually coupled MS platforms, comprised of LC-MS, GC-MS, and CE-MS, could be employed as stand-singly or in tandem to perceive a broader range of metabolites [20]. LC-MS has been employed to isolate and inspect heat-unstable and nonvolatile metabolites and is more perceptive than GC-MS when utilized in tandem MS [21]. However, the main drawback of LC-MS is its limited spectral replicability, which complicates the identification of metabolites using present spectral libraries [22]. Some salmonid investigations were conducted using LC-MS, the next most frequently used analytical method. Especially, this tool was prevalently practical in nutritional and ecotoxicological studies [23].

GC-MS, the 3rd most utilized analytical method in fish metabolomics, was able to separate volatile and heat-stable metabolites. GC-MS is replicable, perceptive, and attains outstanding metabolite parting. Nevertheless, GC-MS is inappropriate for heat-unstable or metabolites at elevated MW. In addition, some sample extracts intended for GC-MS analysis may necessitate derivatization [24]. Young et al. [25] employed GC-MS to validate

the metabolic impacts of severe treatment and anesthesia in salmon. Similar to LC-MS and GC-MS, the CE-MS sensitivity does not involve sample pre-treatment, can utilize minor trials, and can distinguish some metabolites that are not proper for other MS plans. Conversely, for nonpolar compounds, CE hurts from reduced reproducibility and absence of parting [26].

Raman and IR, which are both a type of vibrational spectroscopy, provide data on wider metabolite functional groups and do not tolerate metabolite determination. These tools confuse the understanding of spectra resulting from composite metabolite combinations and afford inferior determination data.

Transcriptomics tools are adapted methods to investigate an organism's transcriptome, collecting the sum of its RNA transcripts. The mRNA acts as a passing intermediate molecule in the information system, while noncoding RNAs perform a wide range of functions [27,28]. A transcriptome releases a picture in time of the total transcripts present in a cell. To isolate RNA, all transcriptomic approaches need RNA to first be extracted from the experimental organism before transcripts can be detailed. RNA extraction methods included mechanical cell/tissue disturbance and RNase with chaotropic salts [29], macromolecules and nucleotide complex interruption, RNA separation from undesired biomolecules counting DNA, and RNA concentration by elution from a solid matrix or precipitation from solution [30].

Transcriptomics methods are extremely similar and need noteworthy computation to generate expressive data for both RNA-Seq experiments and microarrays [31]. These latter, microarray data, were documented as high-resolution images, necessitating feature detection and spectral examination. In addition, numerous short probes that correspond to a single transcript can disclose details about the intron-exon assembly, needing statistical models to control the authenticity of the resulting signal.

RNA-Seq studies can create $>10^9$ of short DNA sequences, which must be aligned to reference genomes including millions to billions of base pairs. RNA-Seq operations are extremely repetitive [32,33]. For every gene, processed count data would be much less comparable to managed microarray intensities. As an illustration, transcriptomics are appropriate for classifying the presence of offal in minced meat by directing the product of transcription, and miRNAs are selected as a potential biomarker to distinguish chicken organs from each other.

Vishnuraj et al. [34] established an innovative miRNA-based approach to detect the occurrence of gizzard, liver, and heart in chicken skeletal meat. This study achieved extensive miRNA sequencing extracted from the dissimilar chicken body parts. In that study, three miRNAs were designated as tissue-specific markers for the gizzard and two for the heart and liver.

2.3.2. Proteomics

In biological organisms or tissues, the proteome includes all expressed proteins at a precise time/space. To categorize and control (i) kinds and (ii) the plenty of proteins, protein/protein communication, the proteins' location in cells, and post-translational change, proteomics employs a variety of techniques [35]. As an example, consider protein translational modifications (PTMs). Three major PTMs predicted on the dbPTM registry are (1) phosphorylation, (2) acetylation, and (3) ubiquitination, which account for more than 90% of all described PTMs. Consequently, each amino acid has at least three diverse PTMs, with Lys having the most (at 15 PTM) [36].

Phosphorylation plays an important role in significant cellular progressions, such as in replication, transcription, environmental stress reactions, cell metabolism and immunology, movement, and apoptosis responsiveness [37]. Phosphorylation is the most investigated PTM, and it is one of the most common kinds of PTM on target proteins, frequently occurring in the cytosol or nucleus [38]. This adaptation could alter the protein's role in a brief term by allostery or by connecting to interaction domains [39]. Biochemically, Ser, Tyr,

Thr, Pro, His, Arg, Asp, and Cys residues are the major target phosphorylation positions; nonetheless, this adaptation mainly occurs on Ser, His, Thr, and Tyr [40].

Acetylation plays a central role in physiological functions, like chromatin stability, protein–protein interactions, cell metabolism, cell cycle command, nuclear transport, and actin nucleation [41]. Actuated via histone acetyltransferase (HAT) and lysine acetyltransferase (KAT) enzymes, acetylation comprised three systems: Nα-, Nε-, and O-acetylation and could happen on Lys, Arg, Ala, Cys, Asp, Gly, Glu, Met, Ser, Pro, Val, and Thr [42]. Likewise, under various stimuli, protein change can speedily control its activity and function [43]. Since protein answers for the principal part of muscle excluding moisture, numerous proteomic modifications could happen throughout the process of conversion from muscle to meat. So, proteomics technology can be applied to assess meat quality biomarkers.

Proteomics tools comprised three stages: (i) 2-dimensional gel electrophoresis, (2) protein chips, and (3) mass spectrometry. This latter is extensively employed in the protein expressions, post-translational change, and subunit-function connections liable for meat quality changes. Nevertheless, it is challenging to achieve precise qualitative analysis only by applying mass spectrometry. To better explain the molecular mechanisms of biochemical routes, other approaches have been united with MS to conduct detailed quantitative proteomics studies. As illustrations, the mixed modes of DDA/DIA data were extensively reported. In this regard, Santos et al. [44] stated the mixed-data acquisition (MDA) strategy aiming to combine DDA and DIA in the same run, which avoids the additional demand for creating distinct registries. Additional marginally innovative targeted methods include parallel reaction monitoring (PRM) and sequential windowed acquisition of all theoretical fragment ion mass spectra (SWATH-MS) [45]. These are typically achieved on a Q-TOF or Q-OT instrument; the fragment ions are sensed with great MS accuracy and resolution as opposed to SRM. PRM differs from SRM because the peptide collection is complete and produce ion selection is not compulsory.

SWATH-MS used DIA mode to combine the benefits of both methods by conveying intense proteome analysis with high authenticity and constancy. In SWATH-MS, for precursor ion selection, an achievement window is intended to cover the entire mass extent in which most of the peptide precursors in a biosample could drop. The precursor ions are consecutively designated over the separation window, exposed to collision, and the entire ion product resulting from every bit of the precursors is transmitted to the detector [46].

In fisheries exploration, gel-based proteomics have been extensively used for investigating numerous features, such as evolutionary biology, environmental surveillance, toxicology, disease, health, and overall proteome outlining [47]. Here, the protein separation on gel was succeeded by (1) removal of the wanted gel band, (2) protein digesting in gel, and (3) protein elucidation by MS. Commonly, proteins are isolated on polyacrylamide gels, and this parting is conducted by 1 or 2-dimensional (1DE, 2DE) electrophoresis [48]. Using 1-DE (10% acrylamide), liver proteomics of rainbow trout against *Aeromonas salmonicida* disease was assessed, and ≈3000 proteins were perceived by means of label-free quantification [49]. On the 2DE, proteins are detached on the basis of isoelectric point (pI) in the 1st dimension and MW in the 2nd one [50]. Attained gels are further marked for conception and evaluation of gel profiling and protein stains through numerous experimental situations. Finally, via bioinformatic programs, stain revealing, quantification, and corresponding with different groups of gels are achieved [51]. Throughout the fasting–refeeding diet, 2DE identified the proteomic modifications in the muscle of Pacu twitch [52]. In addition, 2DE was employed to study authentication, protein biomarker identification for numerous diseases or infections, effects of environmental factors, and toxicological impacts of pollutants [53]. On the other hand, 2D has several disadvantages, like gel alterations and spot standardization concerns. To avoid these matters, 2D-difference in gel electrophoresis (2D-DIGE) was used, which is a further accurate and perceptive technique for quantitative investigation [54]. In DIGE, spectrally solvable shining tags (CyDyes™; Cy5, Cy3, and

Cy2) are used for labeling, and have been used to examine the impacts of some regimes on fish allergenicity and muscle performance, as well as the impact of storage temperature and other quality aspects [55]. Targeted proteomics is an unconventional technique for immunoassay that determines involved proteins and their matching peptides [53]. Here, targeted fish proteins can be noticed with reproducibility, specificity, and accuracy. Multiple reaction monitoring (MRM) was usually employed as a targeted approach, and the SRM was achieved by the QQQ mass spectrometer.

2.3.3. Lipidomics

Lipidomics performed a significant impact in disclosing the lipid groups, abundance, cellular lipid metabolism, and 3-D dispersion, and the flavor metabolomics target volatile compounds and amino acids [56]. As a division of metabolomics, flavoromics and lipidomics implement the same platforms. It is remarkable that volatiles and lipids entail previous extraction, and considering that fish commodities are high in lipids, especially essential fatty acids like omega-3s, these products are predisposed to oxidation throughout production and storage [57].

Communal lipid profile investigations utilize quantitative detectors like (i) flame ionization detectors (FID), diode array detectors (DAD), ultraviolet (UV), and electron light scattering detectors (ELSD), and charged aerosol detectors (CAD) [58]. To isolate and distinguish charged ionized elements in accordance with their m/z ratios, this omic approach applied separation methods such as TLC (thin layer chromatography), GC, and HPLC, which are joint with MS detectors.

Revolutionary lipidomics investigations engaged the direct injection of the lipid mixture on the MS called direct infusion MS (DIMS) [59]. Targeted and untargeted lipidomics are two other approaches that usually include the chromatographic separation of the sample, which simplifies the isobaric molecule resolution. In a targeted study, the goal-mouth is the quantification and identification of a partial variety of lipids [53]. In a targeted study, the MS is arranged to explore a restricted number of transitions into SRM and MRM and, therefore, grow its sensitivity to the designated ions. The handling of lipidomics includes the application of numerous software implements. For example, the LipidSearch package was exploited to examine the lipid level. To identify the specific lipids included in the sample, lipidomic data found from MS analysis were likened to LipidSearch databases [57].

Chemometrics investigation can be achieved by some software programs like MetaboAnalyst (version 6.0), Minitab (version 21), and Orange (version 3.32.0) for the application of PCA and PLS-DA.

The most frequently utilized omics for the examination of the quality control of aquatic food products are represented in Figure 3.

APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; CE, capillary electrophoresis; COSY, correlation spectrometry; DIA-SWATH, data-independent acquisition-SWATH; DIGE, difference gel electrophoresis; EI, electronic impact; ESI, electrospray ionization; FTICR, Fourier transform ion cyclotron resonance; GC, gas chromatography; ICAT, isotope-codedaffinity tag; ITRAQ, isobaric tags for relative and absolute quantification; LC, liquid chromatography; LIT, linear ion trap; LTQ-Orbitrap, linear-quadrupoleion trap-Orbitrap; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; PCR, polymerase chain reaction; Q, quadrupole; QIT, quadrupole ion trap; qPCR, quantitative PCR; QTrap, triple-quadrupole ion trap; Q-TOF, quadrupole-TOF; SILAC, stable isotope labelling of amino acids in cell culture; TOCSY, total correlation spectroscopy; TOF, time-of-flight; TQ, triple quadrupole.

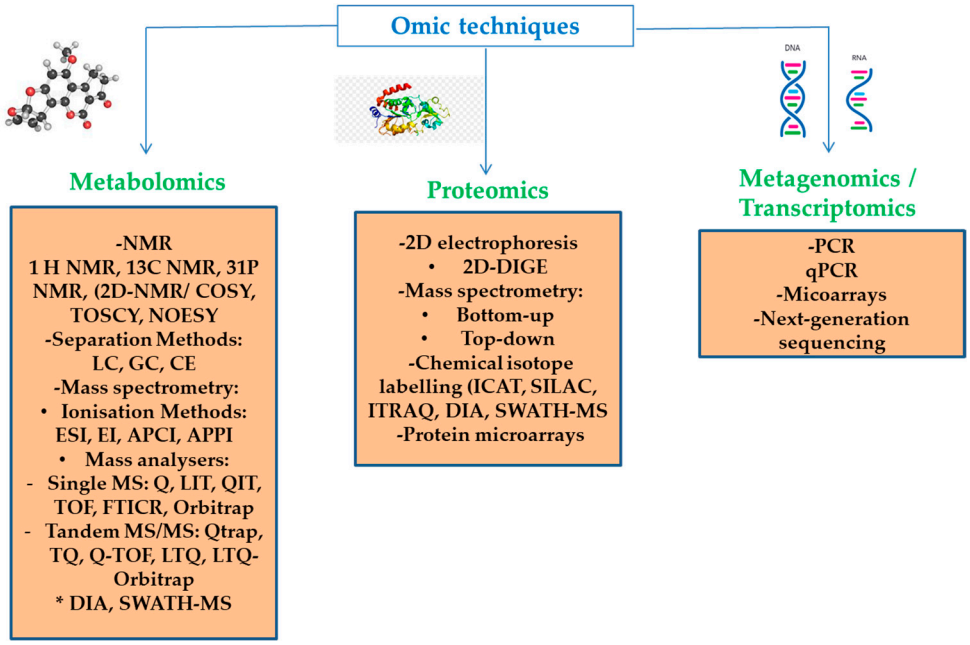

Figure 3. Most frequently used omic tools for the examination of quality and control of aquatic food products.

2.4. Analytical Technologies and Approaches of Omic Technologies

Based on the analytical objectives, omic technologies can be classified into targeted and untargeted. Regarding proteomics, targeted proteomic approaches are suitable for the validation or detection of proteins of interest with high sensitivity, while untargeted approaches are well-suited from the very beginning of biomarker discovery [60]. While integrating proteomic data with other omic technologies, quality and safety of sea food will be ensured [14].

Concerning metabolomics, targeted metabolomic approaches can be used in order to decode the behavior of specific groups of metabolites in relation to defined conditions and emphasize the identification and quantification of preselected metabolites. In untargeted metabolomics, the global metabolite fingerprint is studied [61], and groups of metabolites can be detected without necessarily identifying or quantifying a specific metabolite [44]. According to Chatterjee et al. [61], untargeted metabolomics have many advantages compared to targeted metabolomics, including the combination of targeted and untargeted screening, the finding of a set of discriminatory metabolites, and the retrospective data analysis.

A key technology that should be mentioned and distinguished from the aforementioned metabolomic technology is volatolomics. Volatolomics, a subfield of metabolomics, aims to identify and quantify all volatile metabolites present in a biological system [62]. During fish storage and processing, numerous metabolites like volatile organic compounds (VOCs) are produced [62,63]. Therefore, volatolomics technology brings into being the aroma fingerprint along with flavor profile [62]. Thus, the use of volatolomics provides the identification of the key aroma compounds [64], while the use of metabolomics helps to identify the flavor precursors in seafood [65].

With regard to the lipidomic technologies, targeted approaches emphasize analyzing specific lipid targets, while untargeted approaches intend to analyze the full lipid profile in samples [66].

All the above omic technologies produce tremendous amounts of data, and with the combination of chemometrics, predictive models can be established for food traceability, identification of food adulteration, authentication, safety assessment, and improvement of hazardous substance formation in food processing or food production, and in that manner, improving the food quality [61,66].

Below you can find Tables 1 and 2 describing omic technologies for quality control of aquatic and seafood products.

Table 1. Examples of omic technologies (metabolomics) for quality control of seafood and aquatic products.

Omic Technology	Seafood	Extraction Method	Identification Technique	Data Analysis	Results	Reference
Metabolomics	Sea cucumber (*Apostichopus japonicas*)		LC-MS	ANOVA, PCA, PLS-DA, KEGG, HMDB and the LIPIDMaps database	SMZ stress treatment significantly impacted sea cucumber's metabolism as well as disrupted balance of metabolites like L-threonine, L-tyrosine, neuronic acid, piperine, and docosapentaenoic acid.	[67]
Metabolomics	Chinook salmon (*Oncorhynchus tshawytscha*)		LC-MS		185 endogenous metabolites were detected. Alteration in biochemical pathways in several metabolites, including those that were important for energy generation and utilization after exposure to effluent.	[68]
Metabolomics	Snow crabs (*Chionoecetes opilio*)		CE-TOF/MS	PCA, PLS-DA, HCA	77 target metabolites were identified. Of those, 2-oxovaleric acid, asymmetric dimethylarginine, hypotaurine, and allo-threonine were selected as final biomarkers to unequivocally determine the geographic origin.	[69]
Metabolomics	Hybrid grouper (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*) and golden pompano (*Trachinotus ovatus*)	Warren et al., method	LC-MS/MS	PCA, PLS-DA, KEGG, one-way ANOVA	1678 differential metabolites in positive modes and 1445 differential metabolites in negative modes were investigated in hybrid grouper and golden pompano, respectively. The 16 most differential metabolites were determined as potential biomarkers in different muscle groups including methyldopa, phenylacetate, 4-nitrocatechol, docosahexaenoic acid (DHA), glycerophosphocholine, 9,10–12,13 diepoxyoctadecanoate, 12,13-DHOME, N-acetyl-L-phenylalanine, dimethylglycine, cyclopeptine, thiamine monophosphate, dehydroepiandrosterone, and 15-deoxy-d-12,14-PGJ2.	[70]
Metabolomics	Pacific oyster (*Crassostrea gigas*)		UPLC-QE Orbitrap MS	PLSR, PLS-DA, KEGG	Aspartate, glutamine, alanine, and arginine were the key precursors affecting the flavor profile.	[71]
Metabolomics	Mediterranean mussel (*Mytilus galloprovincialis*)	Cappello et al. method	^{1}H NMR	PCA, ANOVA, Tukey's multiple post-hoc comparisons	Amino acids and energy metabolism was changed, and osmoregulatory processes along with the cholinergic neurotransmission were disturbed.	[72]

Table 1. *Cont.*

Omic Technology	Seafood	Extraction Method	Identification Technique	Data Analysis	Results	Reference
Metabolomics	Mediterranean mussel (*Mytilus galloprovincialis*)		LC-HRMS	PCA	Metabolic profiles showed a strong effect of pharmaceuticals, independently of polyethylene microplastics co-exposure. Nevertheless, polyethylene microplastics impacted metabolic pathways, like neurotransmitters or purine metabolism.	[73]
Metabolomics	Largemouth bass (*Micropterus salmoides*)		UPLC-TripleTOF	PCA, OPLS-DA, KEGG	After being supplemented with 1%, 3%, and 5% of hydrolysis fish peptides 85, 144, and 207 differential metabolites were identified. Differential metabolites were mainly lipids and lipid-like molecules.	[74]
Metabolomics	Mediterranean Mussels (*Mytilus galloprovincialis*)	Weidt et al. method, Shen et al. method	GC-MS	PCA, KEGG, ANOVA	High accumulations of low-molecular-weight polycyclic aromatic hydrocarbons were found in mussels. High body burdens of polychlorinated biphenyls and organochlorine pesticides were only found at mussels from the site close to the river mouth. Some of the metabolic pathways were correlated with the accumulation of polycyclic aromatic hydrocarbons.	[75]
Metabolomics	White leg shrimp (*Litopenaeus vannamei*)		UPLC-QTOF-MS	PCA, OPLS-DA, PLS-DA, KEGG	Pathways of amino acid, glycerophospholipid, and nuclei acid metabolism and ABC transporters in hepa topancreas were significantly disturbed, and 37 metabolites were identified.	[76]
Metabolomics	Chinese shrimp (*Fenneropenaeus chinensis*)	Zhang et al. method	^{1}H NMR	PCA, OPLS-DA, PLS-DA	Glycine and serine could serve as metabolic markers for Cd in *F. chinensis*.	[77]
Metabolomics	Gilthead sea bream (*Sparus aurata*)	Wu et al. method	GC–MS	PCA, ANOVA, PLS-DA	They found 27 differential metabolites distinguished in two groups (14 relative content increased and 13 decreased during storage on ice, respectively).	[78]
Metabolomics	Shrimp (*Penaeus monodon*, *Litopenaeus vannamei*, *Fenneropenaeus indicus*, *Metapenaeus monoceros*, *Pleoticus muelleri*)		LC-MS/MS	PCA, OPLS-DA	For targeted metabolomics, 34 markers were evaluated and 17 of them were considered spicies specific (authentication of species identity). Another 6 significantly different markers were found to differ between geographical origin within the assessed species groups (authentication of geographical origin).	[61]

Table 1. *Cont.*

Omic Technology	Seafood	Extraction Method	Identification Technique	Data Analysis	Results	Reference
Metabolomics	Japanese medaka (*Oryzias latipes*), zebrafis (*Danio rerio*)	Wu et al. method, Cappello et al. method	^{1}H NMR	ANOVA, Dunnett's post-test	The levels of endogenous metabolites were significantly changed ($p < 0.05$) due to imidacloprid exposure.	[79]
Metabolomics	Mussels (*Mytilus galloprovincialis*)	Cappello et al. method, Fasulo et al. method	^{1}H NMR	Student's *t*-test	Significant decrease of all measured amino acids (36% in isoleucine, 24% in leucine, 25% in valine, 32% in arginine, 47% in glutamate, and 67% in glutamine) in the digestive glands of polluted mussels.	[80]
Metabolomics	Mussels (*Mytilus galloprovincialis*)	Alvarez et al. method	HPLC-HRMS	ANOVA, PCA, Tukey's post hoc test	Alterations in amino acids levels (aspartate, phenylalanine, valine, and tryptophan), as well as disturbances in osmotic regulation and energy metabolism were observed. No significant alterations in the enzymatic activities nor in carboxylic acid levels.	[81]
Metabolomics—volatilomics	Pacific oyster (*Crassostrea gigas*)		HS-SPME-GC–MS, SAFE-GC-MS, GC-IMS	PLSR, PLS-DA, KEGG	They detected 15 key odorants (Pentanal, 1-penten-3-ol, hexanal, (E)-2-pentenal, heptanal, (E)-2-hexenal, 4-octanone, (E)-4-heptenal, 3-octanone, octanal, nonanal, 1-octen-3-ol, benzaldehyde, (E)-2-nonenal, and (E, Z)-2,6-nonadienal). Hexanal, (E)-4-heptenal, and (E)-2-pentenal were significantly associated with off-odor; 177 differential metabolites were classified.	[71]
Metabolomics—volatilomics	European seabass (*Dicentrarchus labrax*) and Atlantic salmon (*Salmo salar*)		SPME–GC/MS	ANOVA Tukey's significant diference test	Of those, 22 Aldehydes, 12 ketones, 13 alcohols, 6 esters, and 1 acid found in both fish species; 3-hydroxy-2-butanone, 2,3-butanediol, 2,3-butanedione and acetic acid could be proposed as potential spoilage markers.	[63]
Metabolomics—volatilomics	Low-salt fermented sour fish		HS-SPME-GC/MS		Ethyl acetate, ethyl hexanoate, isoamyl acetate, ethyl butyrate, hexanal, 1-hexadecanal and 2-pentylfuran were the key aroma compounds.	[64]
Metabolomics—volatilomics	Smoked tuna and swordfis	Beltran et al. method and Sales et al. method	GC-EI-MS	PCA, PLS-DA	They identified 11 markers (3-methylcyclopentanone, ethylbenzene, 2-methyl-2-cyclopenten-1-one, 2-methyl-benzofuran, furfuryl alcohol, 2-acetylfuran, acetophenone, guaiacol, 1-hydroxy-2-butanone, 4-vinylguaicol and acetoin)	[82]

Principal component analysis (PCA), orthogonal projections to latent structures discriminant Analysis (OPLS-DA), partial least square discriminant analysis (PLS-DA), hierarchical cluster analysis (HCA), analysis of variance (ANOVA), Kyoto Encyclopedia of Genes and Genomes (KEGG), partial least squares regression (PLSR).

Table 2. Examples of omic technologies (proteomics, lipidomics, and metagenomics) for quality control of seafood and aquatic products.

Omic Technology	Seafood	Extraction Method	Identification Technique	Data Analysis	Results	Reference
Proteomics	Turbot and sea bream	Carrera et al. method	LC-MS/MS	PANTHER software	A total of 1015 peptides associated with virulence factors were identified. A total of 25 species-specific peptides were identified as putative *Pseudomonas* spp. biomarkers.	[83]
Proteomics	Grass carp (*Ctenopharyngodon idellus*)		LC-MS/MS	PCC, KEGG, Bonferroni correction	A total of 1085 proteins were identified, 516 of which indicating a core proteome responsible for Grass carp textural properties.	[84]
Proteomics	Dry-cured squid (*Dosidicus gigas*)	Lin et al. method	LC-MS/MS	ANOVA, Student's *t*-test, SPSS, Duncan's test	A total of 1148 proteins in squid samples during the dry-curing process were identified. Of those, 32 key differentially abundant proteins were found to be correlated with sensory and texture characteristics, including myofibrillar protein, tubulin beta chain, collagens, heat shock proteins and cytochrome.	[85]
Proteomics	Shellfish and fish (*Mercenaria mercenaria*, *Solen strictus*, *Mactra antiquata*, *Solen giandis*, *Musculus senhousei*, *Parabramis pekinensis*, *Carassius auratus*, *Aristichthys nobilis*, *Paphia undulata*, *Perna viridis*, *Pseudocardium sachalinense*, *Panopea abrupta*, *Cypraea cumingii*, *Mactra quadrangularis*, *Saxidomus purpuratus*, *Ctenopharyngodon idellus)*	Zhu et al. method	LC-MS/MS	PRIDE database	Identification of novel proteins and strain-specific proteins in *V. cholerae* isolates recovered from 16 species of consumable aquatic animals; 215 common and 913 differential intracellular proteins, including 22 virulence-associated and 8 resistance-associated proteins were identified.	[86]
Proteomics	Grass carp (*Ctenopharyngodon idellus*)	Dazert et al. method	LC-MS/MS	KEGG	A total of 27 up-regulated and 22 down-regulated phosphopeptides were detected.	[87]
Proteomics	Silver carp (*Hypophthalmichthys molitrix*)	Yu et al. method	LC-MS/MS	SPSS, PCC, Duncan's multiple range test	A total of 43 differentially abundant proteins were detected and involved in muscle contraction, energy metabolism, antioxidant defense, protein turnover, etc. Those differentially abundant proteins were selected as potential proteomic markers to trace the accelerated textural softening in stunning-stressed fillets.	[88]

Table 2. *Cont.*

Omic Technology	Seafood	Extraction Method	Identification Technique	Data Analysis	Results	Reference
Lipidomics	Basa catfish (*Pangasius bocourti*) and Sole fish (*Cynoglossus semilaevis Gunther*)	Matyash et al. method	UHPLC-QE Orbitrap MS	PCA, OPLS-DA, VIP	A total of 779 lipid molecules from 21 lipid subclasses were detected. Significant differences between basa catfish and sole fish were observed. A total of 165 lipid molecules were screened out as discriminative features and authentication/distinction between the two fish spieces was achieved.	[66]
Lipidomics	Dried shrimps (*Penaeus vannamei*)	Li et al. method	UPLC-MS/MS	ANOVA, PCA, PLS-DA	A total of 790 lipid molecular species belonging to 7 main classes were identified. Glycerophospholipids (GPs), glycerolipids (GLs), sphingolipids (SPs), phosphatidylcholine (PC), phosphatidylethanolamine (PE), triglyceride (TG) and diglyceride (DG). A total of 163 differential lipid molecules were screened and 18 differentially abundant lipids were selected as potential biomarkers of lipid oxidation in dried shrimps.	[89]
Lipidomics	Carp (*Cyprinus carpio*)		LC–MS/MS	ANOVA, PCA, KEGG Duncan's multiple comparison tests	A totla of 261 differentially expressed lipid metabolites were identified, including phosphatidylethanolamine (PE), phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylserine (PS), and cardiolipin (CL).	[90]
Metagenomics	European sea bass (*Dicentrarchus labrax*)	Cocolin et al. method	16S rRNA gene sequence	Clustal Omega algorithm	A total of 10 differentiated groups of unknown microbiota was identified. *Ps. Cryohalolentis* was the most dominant at the beginning of fish shelf-life, while *Ps. glacincola* increased and dominated at the time of the sensory minimum acceptability (day 14) and rejection (day 16).	[91]

Principal component analysis (PCA), orthogonal projections to latent structures discriminant Analysis (OPLS-DA), partial least square discriminant analysis (PLS-DA), analysis of variance (ANOVA), Pearson coefficient correlation (PCC), statistical package for the social sciences (SPSS), Kyoto Encyclopedia of Genes and Genomes (KEGG), variable importance in projection (VIP), proteomics identifications database (PRIDE).

3. Omics for Detection of Pathogens and Spoilage Microorganism in Aquatic Food Products

Fish spoilage begins promptly when a fish dies and is a consequence of three types of deteriorative changes. The first is autolysis caused by fish enzymes; the second is bacterial decomposition, which starts while autolysis is proceeding; and finally, the third is the

chemical changes, which can produce rancid odors and flavors [92]. Chilling can slow down this process and prolong the shelf life of aquatic food products [93], but under particular storage conditions there are Specific Spoilage Organisms (SSOs) that can be dominated against others and produce metabolites serving for the development of off-flavors and off-odors in aquatic food products, causing their sensory rejection [63].

Omic technologies are found to be effective in the quality control of perishable foods. More specifically, it has been stated for years that proteomics has been successfully applied for the identification and detection of pathogenic and spoilage microorganisms [94] and, till now, has been successfully used for the identification of proteins related to bacterial infection [95]. Moreover, according to Yang et al. [96], metabolomic analysis using HPLC/MS was performed in shrimp (*Marsupenaeus japonicus*) intestinal contents. Data indicate clear changes in that the metabolic profiles of shrimp intestines after *V. parahaemolyticus* infection. More specifically, 39 metabolites changed significantly after the infection.

Nevertheless, it is essential and extremely important to distinguish the existence or nonexistence of microorganisms in food, as well as nutrients like fats, proteins, preservatives, or additives that are related to the microbial growth and stability [97]. The molecular processes are adequately reflected by the proteomics techniques, and as a result, proteomics could be a powerful tool for the characterization of proteomes of any aquatic food products and the identification of molecular markers for their quality and safety [14]. Several years ago, during the processing, preservation, and storage of seafood, proteomic technologies were used for the identification of common pathogenic bacteria like *Acinetobacter*, *Aeromonas*, *Bacillus*, *Carnobacterium*, *Clostridium*, *Listeria*, *Photobacterium*, *Pseudomonas*, *Stenotrophomonas*, *Shewanella*, *Staphylococcus*, *Vibrio*, *Campilobacter*, *Staphylococcus*, and *Enterobacter* [98–100]. Since then, not only proteomics is becoming increasingly popular in the characterization of the virulence of microbial pathogens during the processing and storage of aquatic food products, but also next generation sequencing (NGS) shows the potential and usefulness for the determination of microflora related to foodborne diseases and deterioration of seafood [3,83,86]. By using metagenomics technologies, prediction for the presence of pathogens and spoilage microorganisms along with the characterization of unknown microbiota can be accomplished and determined from modifications that have been observed in entire microbial communities. Metagenomics technologies could improve the safety and quality of aquatic food products [101].

4. Metabolomics for Metabolite Detection in Aquatic Food Products

4.1. Muscle Quality and Taste

In aquatic food products, quality assurance is a significant aspect and has gained special attention. For instance, metabolomics approaches could be engaged for fish post-harvest quality control [102–104]. In these studies, investigations on fish quality established the major targeted metabolomics as LC-MS, HR-MAS-NMR, GC-MS, and DART-HR-MS. In salmonids, these methods were used to quantify total lipids, omega-3, DHA, and EPA content [23,105], validate farming of fish, allocate origin [106–108], classify K-value and trimethylamine nitrogen (TMA-N) [109], and types of lipids during cold storage, which are indicators of quality and freshness [110].

Ghidini et al. [111] implemented a near-infrared spectroscopic to authenticate *D. labrax* fillets, which were dependent on their cultivation system (wild-caught vs. farmed), farming system, and geographic source. Statistical analysis matched to spectral AAs and peptide absorption and region could illustrate correctly (at 100%) cultivated from wild fish and authenticated fish at 67% (general), 80% semi-intensive, and 100% (intensive growing). Collected from different European regions, the fatty acids of *D. labrax* were effectively distinguished by spectral data [111]. This investigation stressed the capacity of metabolomics for rapidly determining the origin, distinguishing wild–harvested from type of farm, and battling fraudulent substitution and mislabeling of fish products [112,113]. Gribbestad et al. [114] developed a ^{1}H-NMR approach for *S. salar* authentication and classi-

fication. The platform permitted to identify hypoxanthine, individual AAs, FAs, lactate, and anserine, which are indicative of nutritional profile and overall muscle quality. Based on targeted systems employed to differentiate between wild and farmed salmon, ^{1}H-NMR was utilized to investigate the impact of salmon feed on wild *Pollachius virens* (saithe) [115]. The metabolomics of muscle and liver tissues exposed high levels of glutamine, alanine, lactate, and glutamate in wild saithe caught around salmon farms. In addition, enclosure-scavenging wild saithe have reduced levels of glucose and choline compared to control wild fish. This reproduced the variation in the metabolic tissue profile between the two groups. The authors reported that the raised AAs and lactate concentrations were due to lactic acid anaerobic fermentation as a result of inadequate O_2. The ^{1}H-^{13}C-NMR was employed to control the postmortem spoilage in *S. salar* fillets kept at 0 and 4 °C [116]. The NMR profile of fillet displayed metabolites, suggesting an autolytic and microbial spoilage below chilled conditions [117]. At 4 °C, fillets presented an earlier reduction in glycine and glutamate levels, indicating bacterial degradation. By virtue of the autolytic spoilage, His, β-Ala, and 1-methyl-His analyses were developed at 4 °C rather than at 0 °C. Fillets retained more trimethylamine oxide (TMAO) to trimethylamine (TMA) than samples stored at 0 °C. The Tyr and Lys breakdown engendered a specific intensification of malodourous tyramine and cadaverine in fillets stored at 4 °C [116]. Metabolomics detected TMA as a spoilage biomarker, while a reduction in glucose followed by an increase in organic acid levels signaled the microbial sugar fermentation. In this study, photobacterium, the principal bacterial genus, could convert tissue TMAO to raised TMA levels and exploit glucose as a C source to progress the spoilage throughout *S. salar* cold storage of vacuum-packed [118]. Shumilina et al. [119] classified raw from frozen-thawed *S. salar* fillets. In this study, ^{1}H-NMR metabolomics differentiate these two different sample fillets. The aspartate increase in freezing and unfrozen fish was provoked by the elevation of activity and leakage of aspartate aminotransferase (AAT), which shifts an amine group from 2-oxoglutarate to L-aspartate.

Metabolomics was also employed to estimate the fish taste features. As an illustration, in fish meat, robust relations were established among "sourness" and Lys, "irritant" and Ala and Phe, "saltiness" and pantothenic acid, and "umami" and creatinine and His [120]. Phosphoric acid was recognized as an applicant indicator assessing changes in the taste of four species of fish [121]. It should be pointed out that metabolomics is extensively employed in assessing freshness and recognizing freshness-related biomarkers of fish meat like tuna, yellowtail, gilthead sea bream, tilapia, and komatsuna [122,123].

4.2. Chemical Contaminants

Aquatic food products are one of the most important cardioprotective food groups with beneficial effects on human health because of the unsaturated fatty acids and primarily polyunsaturated fatty acids, which are found in abundance [1]. Nevertheless, there are also potential hazards since it may contain specific industrial or environmental contaminants, such as pathogens, dye residues, marine toxins, and environmental pollutants (including heavy metals). For example, methylmercury or persistent organic pollutants (POPs) like DDT, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) can have hazardous effects on human health like immune toxicity, reproductive toxicity, neurotoxicity, gene mutations, and DNA methylation after long-term exposure at low levels. They are also known to have negative economic impacts [75,97,110]. Measuring and documenting these effects can function as an indicator of environmental conditions and as an aquatic animal health surveillance, consequently helping to protect an important source of human food [124].

According to the Food and Agriculture Organization (FAO), maximum levels of environmental chemical contaminants have been established for a number of compounds (Table 3).

Table 3. Environmental chemical contaminants. Tolerances and critical limits in fish and fish products [125].

Substance	Maximum Levels US (ppm)	Maximum Levels EU (mg/kg Wet Weight)	Food Commodity
Arsenic	76–86		Molluscs, crustaceans
Cadmium	3–4	0.05–1.0	Fish, molluscs
Lead	1.5–1.7	0.2–1.0	Fish, molluscs
Methyl mercury	1.0	1.0	All fish
PCB	2.0		All fish
DDT, TDE	5.0		All fish
Diedrin	0.0		All fish
Dioxin		0.000004	All fish

PCB: polychlorinated biphenyls, DDT: Dichlorodiphenyltrichloroethane, TDE: 2,2′-Thiodiethanol.

In order to guarantee a high level of protection for human health, a thorough legal framework was developed. The European Parliament and the Council of the European Union have adopted Regulation (EU) No. 1379/2013 [126], according to which fishery and aquaculture products should comply with applicable rules on food safety and hygiene and provide clear and comprehensive information on the origin and the method of production. Moreover, both the commercial and scientific name are required. Additionally, national authorities should make full use of available technology, including DNA testing, in order to deter operators from falsely labeling catches in order to protect consumers.

Undoubtedly, omic technologies could be a complimentary analytical tool to existing analytical DNA-based methods. Proteomic technologies, for example, can provide comprehensive information of the whole system enclosing co-transcriptional modifications, giving the ability of a clear and deep understanding of proteins' composition at a molecular level [127]. According to Kumar et al. [97], studies have shown that environmental pollutants and toxic metals cause changes in the proteome of seafood, indicating a detrimental impact on aquatic food product quality. More specifically, the alteration of physio-metabolic response and the activation of proteins and metabolites are associated with the detoxication and degradation of structural proteins degradation, such as actin, myosin, and desmin.

Metabolites could also be reliable markers of the physiological and biochemical status in organisms. Thus, the metabolic abnormalities could be used to elucidate and understand the physiological responses and biochemical reactions provoked by exogenous factors like pollutants [77]. Metabolomic technologies like mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy could be used in order to detect these minor metabolic abnormalities in stress-exposed tissue samples and, as a result, to interpret the aforementioned physiological responses and biochemical reactions [77,79,80,128].

In the framework of the Lu et al. [77] study, NMR metabolomic technology was used for detecting metabolic changes and determining the metal pollution-induced biological effects in the muscle tissues of shrimp (*Fenneropenaeus chinensis*). According to Wu et al. [74], metabolomics analysis in largemouth bass (*Micropterus salmoides*) based on copper (Cu) exposure revealed that after supplemented with 1%, 3%, and 5% of hydrolysis fish peptides, 85, 144, and 207 differential metabolites were identified in these three different supplemented groups, respectively. Research findings indicate that supplementation with 1%, 3%, and 5% hydrolysis fish peptides significantly reduced the copper bioaccumulation in largemouth bass. Key findings highlight that the differential metabolites were mainly lipids and lipid-like molecules, which were closely related to lipid metabolism pathways. Moreover, metabolomic analysis in shrimp (*Litopenaeus vannamei*) that were exposed to ammonia and performed by GC-MS showed that amino acid metabolism was significantly changed, nucleotide metabolism was significantly decreased, and finally that lipid metabolism was disrupted [129].

De Marco et al. [72] used ^{1}H NMR-based metabolomics to thoroughly explore the metabolic disorders caused by microplastics in Mediterranean mussel (*Mytilus galloprovin-*

cialis) gills. Data indicated that amino acid and energy metabolism was changed, and osmoregulatory processes along with the cholinergic neurotransmission were disturbed. With the same metabolomic technology, Zitouni et al. [130] investigate the effects of environmental microplastics on commercial fish *Serranus scriba* (Linnaeus, 1758). More specifically, the distinct change patterns of metabolites in the liver of *Serranus scriba* were identified, and a total of 36 metabolites primarily involved in energy, amino acid, and osmolyte metabolism were significantly affected. In another metabolomic study, combined effects of polyethylene microplastics and two pharmaceuticals (citalopram and bezafibrate) on Mediterranean mussels (*M. galloprovincialis*) were explored. Following the LC-HRMS analyses, findings suggest that the metabolic profiles of mussels showed a strong effect of pharmaceuticals, independently of polyethylene microplastics co-exposure. Nevertheless, polyethylene microplastics in themselves impacted metabolic pathways, like neurotransmitters or purine metabolism [73].

Recently, effects from the exposure of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) to effluent from an urban wastewater treatment plant were investigated. LC-MS metabolomic technology was used, and research findings indicate alteration in biochemical pathways in several metabolites, including those that were important for energy generation and utilization. With regard to the altered pathways mentioned, it is worth noting that they are crucial for fish health and could become an early indicator of potential negative impact on growth, reproduction, behavior, and immune function [68].

4.3. Veterinary Drug Residues

Veterinary medicines in breeding animals are widely used when treatment is required in order to prevent or cure a disease. These medicines contain active pharmaceutical ingredients (APIs), and it is impossible not to leave residues in the food from cured animals. According to EU regulations, there is a control plan covering four discrete areas. The first and the second are for substance groups APIs listed in Groups A and B, respectively [131], while the third and the fourth are for pesticides and other contaminants to which animals have been exposed. Without exception, food residue levels should not harm the consumer. Within the framework of this control plan, there should be minimum control frequencies for food placed on the Union market from a third country. Minimum control frequencies for aquatic food products are listed below (Table 4).

Table 4. Minimum control frequencies in the control plan for aquatic food products placed on the Union market [132].

Aquatic Food Products	Control Frequency
Unprocessed fishery products * (excluding crustaceans)	Minimum 1 sample per 700 tonnes of annual production of aquaculture for the first 60,000 tonnes of production and then 1 sample for each additional 2000 tonnes For wild caught fishery products, the number of samples is to be determined by each Member State according to the level of production and the problems identified
Crustaceans and bivalve molluscs	The number of samples is to be determined by each Member State according to the level of production and the problems identified
Animal and marine fats and oils	The number of samples is to be determined by each Member State according to the level of production and the problems identified

* Fishery products as defined in EC Regulation No. 853/2004, and refers to all seawater or freshwater animals (except for live bivalve mollusks, live echinoderms, live tunicates, and live marine gastropods, and all mammals, reptiles, and frogs), whether wild or farmed, and including all edible forms, parts, and products of such animals.

It is also important to note that in EU Regulation No. 37/2010 [133], active pharmaceutical ingredients (APIs) and their classification regarding maximum residue limits (MRLs) for residues are listed in the Annex. Only data on fish were selected from the aforemen-

tioned Annex and gathered together in Table 5 below. It should be noted that prohibited substances such as chloramphenicol, chlorpromazine, colchicine, dapsone, dimetridazole, metronidazole, nitrofurans (including furazolidone), and ronidazole are also set out in the Annex with their MRL, which cannot be established.

Table 5. Active pharmaceutical ingredients (APIs) and their classification regarding maximum residue limits (MRLs) for residues [133].

APIs	MRL	Target Tissues	Other Provisions (According to Article 14(7) of Regulation (EC) No 470/2009)	Therapeutic Classification
Amoxicillin	50 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Ampicillin	50 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'	Anti-infectious agents/Antibiotics
Azagly-nafarelin	No MRL required	Not Applicable	Not for use in fish from which eggs are produced for human consumption.	No Entry
Azamethiphos	No MRL required	Not Applicable	No Entry	Antiparasitic agents/Agents against ectoparasites
Benzylpenicillin	50 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Bronopol	No MRL required	Not Applicable	No Entry	No Entry
Chlortetracycline	100 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Cloxacillin	300 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Colistin	150 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Danofloxacin	100 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Deltamethrin	10 µg/kg	Muscle and skin in natural proportions.	No Entry	Antiparasitic agents/Agents against ectoparasite
Dicloxacillin	300 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Difloxacin	300 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics

Table 5. *Cont.*

APIs	MRL	Target Tissues	Other Provisions (According to Article 14(7) of Regulation (EC) No 470/2009)	Therapeutic Classification
Doxycycline	100 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Emamectin	100 µg/kg	Muscle and skin in natural proportions	No Entry	Antiparasitic agents/Agents acting against endo- and ectoparasites
Enrofloxacin	100 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Eprinomectin	50 µg/kg	Muscle and skin in natural proportions	No Entry	Antiparasitic agents/Agents acting against endo- and ectoparasites
Erythromycin	200 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Florfenicol	1000 µg/kg	Muscle and skin in natural proportions	Not for animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Fluazuron	200 µg/kg	Muscle and skin in natural proportions	No Entry	Antiparasitic agents/Agents (acting) against ectoparasites
Flumequine	600 µg/kg	Muscle and skin in natural proportion	Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Gentamicin	50 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Imidacloprid	600 µg/kg	Muscle and skin in natural proportions	No Entry	Antiparasitic agents/Agents against ectoparasites
Isoeugenol	6000 µg/kg	Muscle and skin in natural proportions	Not Applicable	Agents acting on the nervous system/Agents acting on the central nervous system
Lincomycin	100 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Lufenuron (RS-isomers)	1350 µg/kg	Muscle and skin in natural proportions	No Entry	Antiparasitic agents/Agents (acting) against ectoparasites
Neomycin (including framycetin)	500 µg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics

Table 5. *Cont.*

APIs	MRL	Target Tissues	Other Provisions (According to Article 14(7) of Regulation (EC) No 470/2009)	Therapeutic Classification
Oxacillin	300 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Oxolinic acid	100 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Oxytetracycline	100 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Paromomycin	500 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Praziquantel	20 μg/kg	Muscle and skin in natural proportions	No Entry	No Entry
Spectinomycin	300 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Sulfonamides (all substances belonging to the sulfonamide group)	100 μg/kg	Muscle	The combined total residues of all substances within the sulfonamide group should not exceed 100 μg/kg. For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Chemotheurapeutics
Tetracycline	100 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics
Thiamphenicol	50 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Tilmicosin	50 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
Tosylchloramide sodium	No MRL required	Not Applicable	For water-borne use only.	Not Entry
Tricaine mesilate	No MRL required	Not Applicable	For water-borne use only.	Not Entry

Table 5. *Cont.*

APIs	MRL	Target Tissues	Other Provisions (According to Article 14(7) of Regulation (EC) No 470/2009)	Therapeutic Classification
Trimethoprim	50 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'. Not for use in animals from which eggs are produced for human consumption.	Anti-infectious agents/ Chemotheurapeutics
Tylosin	100 μg/kg	Muscle	For fin fish the muscle MRL relates to 'muscle and skin in natural proportions'.	Anti-infectious agents/Antibiotics

Active pharmaceutical ingredients (APIs), maximum residue limits (MRLs).

According to the EU Annual Report for 2022 [134] on the results from the monitoring of veterinary medicinal product residues, the minimum requirements for the number of samples to be taken were not fulfilled. More specifically, the samples that were analyzed were lower than 50% of the target minimum sampling frequency.

In the light of physiological abnormalities observed, metabolomic strategies have been provided in order to combat illegal drug abuse in aquabreeding. Therefore, metabolomic technologies can be used successfully for the characterization of those complex biological samples, as it is one of the promising omic tools that can produce the chemical pattern or fingerprint of the medicinal treatment. The specificity of the metabolomic techniques through the use of mass spectrometry (MS) sets the stage for the identification of the markers as metabolomics prepares and clears the path for the interpretation of their chemical structures [110]. Numerous studies have been applied using metabolomic techniques in order to study the effect of antibiotic treatment in aquaculture products, and many more are underway.

For instance, according to Serra-Compte et al. [81], metabolomic analysis of sulfamethoxazole antibiotic (SMX) and its metabolites in mussels (*M. galloprovincialis*) took place using the HPLC-HRMS-based method. In that study, alterations in amino acid levels (aspartate, phenylalanine, valine, and tryptophan) as well as disturbances in osmotic regulation and energy metabolism were observed. No significant alterations in the enzymatic activities nor in carboxylic acid levels were shown.

As per a recent study, metabolomics analyses using LC-MS were performed in order to investigate toxicity effects in the intestine of sea cucumber (*Apostichopus japonicas*) because of sulfamethoxazole (SMZ) stress treatment at different concentrations. Considering the results, the aforementioned SMZ stress treatment significantly impacted sea cucumber metabolism and disrupted the balance of metabolites like L-threonine, L-tyrosine, neuronic acid, piperine, and docosapentaenoic acid. The frequently detected antibiotic in the environment, SMZ, triggered the imbalance of metabolites, causing possible inflammatory responses and inhibition of the growth that possibly negatively affected the normal activities of aquatic organisms [67]. Previous research has confirmed that the SMZ stress can induce metabolic disorders in aquatic organisms [135–137].

In closing, the veterinary drugs that are identified predominantly in aquacultured fish include quinolones and fluoroquinolones, chloramphenicol, nitrofurans, sulfonamides, tetracyclines, as well as macrolides [124].

5. Omics in Aquatic Food Product Traceability

The traceability of aquatic products is a combination of three units of information. The first is the commercial identity or authentication, the second is the production method (if it is wild caught or farmed, adulteration of ingredients), and the third is the geographical origin [61].

From an authentication and adulteration point of view, proteomic technology is fit for purpose since it is effective for the commercial identity of the species and detecting adulteration of ingredients [61,127,138,139], while for geographical origin discrimination, metabolomic technology is successfully used [69,140]. Furthermore, lipidomic technology is successfully used for authentication purposes of fish samples. Through the analysis of lipid profiles and using statistical tools, significant differences in the lipid fingerprints among the aquatic food products could be revealed, providing valuable insights into seafood authentication [66].

6. Proteomics and Seafood

An alternative to DNA is offered by proteomics used in fish species authentication in pure and mixed forms of seafood [141–146].

Authentication of species origin of foods can take place by targeted and untargeted approaches in bottom-up proteomics [146]. The target of proteomic methods is species-specific peptide markers aiming at the detection and quantification of species [141–143]. These methods have been effective in identifying adulteration of Atlantic salmon (*S. salar*) with rainbow trout (*Oncorhynchus mykiss*) [147] and can differentiate fish species from the Merlucciidae family with protein factions of parvalbumin [94].

Varunjikar et al. [145] examined twenty-nine fish species from the North Sea, including individual fish, laboratory-prepared mixtures, and commercial products. Authentication of fresh fish species and fish cakes and burgers (processed ones) was carried out using the spectral library approach.

Tandem mass tags (TMTs) and isobaric tags for relative and absolute quantification (i-TRAQ) constitute some of the well-known proteome detection technologies. TMT technology allows simultaneous labeling analysis of 16 samples and forms a peptide in vitro labeling technology using a TMT reagent [148].

Some of the physicochemical properties investigated in swimming crab (*Ovalipes punctatus*) during cold-chain transportation included sensory scores, water-holding capacity (WHC), glucose (GLU) content, catalase (CAT) activity, and urea nitrogen (UN) content. Tandem mass tag (TMT)-based proteomic analysis examined protein alterations as reported by Du et al. [149]. Accumulation of metabolites in crabs was reported by the regulated purine nucleoside metabolic and nucleoside diphosphate-related processes. Moreover, increasing changes in UN content were depicted.

An advanced, high-throughput, and high-precision proteomic technique is depicted by data-independent acquisition (DIA). Segmentation of the full mass spectrometry scan range into multiple windows and comprehensive detection of all ions within each window is the characteristic of DIA, ensuring no protein information is overlooked [150].

Texture-associated biomarkers were selected by Teng et al. [151] via DIA-based proteomics. Extended chilled and iced storage showed a significant decline in texture and moisture characteristics. Isobutyryl-CoA dehydrogenase, mitochondrial, and [phosphatase 2A protein]-leucine-carboxy methyltransferase represented the most significant texture-associated biomarkers for chilled fish. Another biomarker for iced fish was shown to be Staphylococcal nuclease domain-containing protein 1.

The preservation effect of slightly acid electrolyzed water (SAEW) combined with grape seed extract (GSE) improved shrimp quality, as reported by He et al. [152]. This combination treatment effectively inhibited the growth of spoilage bacteria in shrimp, including *Aeromonas*, *Pseudomonas*, *Shewanella*, and *Enterobacteriaceae*. The proteomic analysis was conducted by LC/MS analysis on a Q-TOF/MS (TripleTOF 6600, Sciex, Framingham, MA, USA) coupled with an Eksigent NanoLC-425, with a total of forty-seven different abundant proteins (DAPs) identified and quantified.

In addition, high-throughput and large-scale protein analysis finds gel-based proteomics unsuitable [153] and appears as labor-intensive and time-consuming, thus making gel-free workflow in the proteomics field [154] significant. Matrix-assisted laser desorption/

ionization-time-of-flight (MALDI-TOF) mass spectrometry has been proposed as a rapid and reliable method for fish authentication shifting to a protein fingerprint [155,156].

Differential peptides [157,158], which can be used as potential biomarkers for species discrimination [159], can be determined via the analysis of the variations in amino acid sequences. Species-specific peptides can be detected by Q-Trap mass spectrometry or triple quadrupole mass spectrometry through multiple reaction monitoring (MRM) screens showing high sensitivity, selectivity, reproducibility, and quantitative accuracy [160]. The "gold standard" for targeted quantitative proteomes is the definition of MRM [161], despite prior required information on MRM transitions.

Separation and detection of the peptides derived from trypsin digestion have been carried out by ultra-high-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-Q-TOF/MS) in data-dependent acquisition (DDA) mode as described by Hu et al. [162]. Ninety-three potential species-specific peptides were found.

Multiple reaction monitoring (MRM) mode based on a Q-Trap mass spectrometer was employed for detection specificity. The results showed that three species-specific peptides were possibly those of tuna species (*Katsuwonus pelamis*, *Thunnus obesus*, and *Thunnus albacores*).

Proteomics for Detection of Pathogens and Spoilage Microorganism in Seafood

Application of new techniques for the detection and identification of pathogenic bacteria present in fish aids in the prevention of seafood spoilage but also in the assessment of the risks involved in human consumption of the fish [163,164].

Liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS/MS) could characterize toxins, virulence factors, and other proteins from different bacterial species in several foods. This technique has been employed to identify peptides related to antibiotic resistance, antimicrobial production, virulence factors, and genus-specific peptides, including two specific peptides to the species *S. dysgalactiae* from different strains of *Streptococcus* spp. associated with bovine mastitis [165]. Shotgun proteomics have been applied in *Listeria* spp. [166], *Enterococcus* spp. [156], and histamine-forming and other biogenic amine-producing bacteria present in seafood [163,167].

Eighteen *Pseudomonas* strains, isolated from fish products, have been characterized using shotgun proteomics, as reported by Abril et al. [83].

1015 non-redundant peptides were found to be related to virulence factors. Putative *Pseudomonas* spp. biomarkers were identified as additional 25 species-specific peptides.

A shotgun proteomic technique using a high-accuracy mass spectrometer (Orbitrap XL equipment) has been reported by Abril et al. [168] to characterize and compare analyses of the 11 strains of Gram-negative spoilage bacteria identified in seafood. A total of 773 peptides were characterized as virulence factors that participate in bacterial pathogenesis, while 14 peptides were defined as biomarkers.

Finally, Fan et al. [169] used tandem mass tag quantitative proteomics and non-targeted metabolomics to elucidate the associated mechanism of hexanal, a phytochemical, against *Vibrio parahaemolyticus*. A total of 572 differentially expressed proteins (DEPs) and 241 differential metabolites (DMs) were identified in hexanal-treated *V. parahaemolyticus*. They found that the structure and function of cell membranes were damaged by hexanal, which also inhibited nucleotide metabolism and acted as an antibacterial agent in the seafood industry.

In the same context, the antimicrobial mechanism of natural compounds has been elucidated as reported by [170–172].

7. Lipidomics and Seafood

Lipidomics is the specific analysis that can be used for the detection of species, geographical origin, and freshness. Detection of contaminants such as heavy metals and persistent organic pollutants in fish products can also be achieved [57,173].

The high percentage of lipids, including both essential fatty acids such as omega-3 and non-essential fatty acids, in the fish and seafood industry makes lipidomics very important nowadays [174].

An abundance of long-chain omega-3 fatty acids like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is shown in fish fillets with potential health benefits ranging from lowering cardiovascular disease risk and stroke to certain types of cancer [175,176]. It has been reported that the lipid content of fish fillets might be affected by fish species, age, diet, and environment [177–179].

Omega-3 fatty acids, representing a convenient source of lipids, might also come from canned fish, such as tuna and salmon, stored for long periods of time [180,181]. Of course, we should be careful since risk might occur from the change in lipids of fish during canning and hence the formation of trans-fatty acids, potentially harmful compounds, and possible carcinogens [182].

Other harmful compounds can also be formed, such as polycyclic aromatic hydrocarbons (PAHs), by smoking fish (salmon and cod), thereby affecting the lipid content of fish [183]. Of course the risks are outweighed by the benefits outlined as richness in omega-3 fatty acids and other essential lipids, as reported by Bienkiewicz et al. [184].

Another fish, high in lipids with a distinct flavor and texture, is caviar, or sturgeon roe. Its high lipid content with specific variations is affected by the species of sturgeon and the method of processing. Hence, as reported by Farag et al. [185], species that are high in oleic acid or omega-3 fatty acids can be detected.

Surimi, a type of fish product made of minced fish and other ingredients with varying lipid content, is affected by the species of fish used and the method of processing. Surimi products can be either low in lipids or high in lipids with a good source of omega-3 fatty acids and can also be found with this technique [186,187].

Lipidomics can also determine the fatty acid composition of fatty and lean fish products. Other significant factors affecting the fatty acid composition include fish species, feeding habits, and habitat, as well as processing [188].

Lipidomics can also be employed towards the assessment of the oxidative stability of seafood. The formation of aldehydes and peroxides in fish products with resulting off-flavors, rancidity, and other quality defects is the result of lipid oxidation [189–191]. Moreover, lipidomics contributes to the quantification of oxidation-related compounds in fish products and determining their oxidative stability [192]. Lipidomics can also assess the effect of cooking, smoking, and storage on the composition and lipid content of fish products [193]. It also works towards the optimization of processing conditions for enhancement of quality and nutritional quality of fish products.

In addition, lipidomics determined changes in lipid composition of tilapia fillets either in raw, steamed, boiled, or roasted forms and outlined the process with the best lipid composition [194].

Fish freshness changes could result from freshness research technology based on omics, as revealed by Chen et al. [195]. The number of screened proteins is too much as far as proteomics is concerned [196,197], and freshness is not related to screened proteins [198].

Rapid evaporative ionization mass spectrometry (REIMS), followed by real-time lipidomic analysis, can generate specific lipid fingerprint profiles [127,199,200]. REIMS and lipidomic analysis outlined the lipid oxidation characteristics in salmon after simulation of cold chain interruption as reported by Yin et al. [201].

Air-dried salmon lipidomic fingerprinting at different temperatures by machine learning (ML) methods (neural networks, support vector machines, ensemble learning, and naïve bayes) was investigated by Song et al. [202]. They discovered that glycerophospholipids (GPs), glycerides (GLs), and sphingolipids, are differential expression metabolites (DEMs).

The common octopus *Octopus vulgaris* has become one of the most prized halieutic resources in the EU [203] due to its palatability and richness in omega-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3). It also has a health and nutritional biochemical profile and is rich in

omega-3 PUFA and other lipids [204]. Using a high-resolution mass spectrometry HILIC-LC-MS lipidomics approach, the polar lipidome of the arm muscle of *O. vulgaris* was determined by Gaspar et al. [205]. The main phospholipids were phosphatidylcholine and phosphatidylethanolamine, while the main sphingolipids identified were phosphonolipid CAEP and ceramides. Moreover, a high proportion of EPA, DHA, and several other very-long-chain fatty acids (24:5, 24:6) esterified in the polar lipid were detected.

Xu et al. [206] employed lipidomics and GC-ion mobility spectrometry (IMS) and investigated the correlation between flavor-active compounds and 16 differential lipids in composite surimi. They found that flavor formation was linked with triglyceride degradation and phosphatidylcholine biosynthesis. Fang et al. [207] explored the changes in lipids of fish during frozen storage using lipidomics and found that differences between fresh fish and frozen fish might occur due to lipid side-chain modifications and lipid decomposition during long-term storage.

Liu et al. [208] employed untargeted lipidomics and ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-Q-Exactive Orbitrap mass spectrometry) along with multivariate statistics (principal component analysis (PCA)) and partial least squares-discriminate analysis (PLS-DA) to determine the lipid profiles of oyster *Crassostrea gigas* from the Yellow Sea (YS), East China Sea, and South China Sea (SCS). The results elucidated that lipid content, composition, and lipid molecular profiles were greatly affected by geographical differences. Hence, geographical origin traceability can be based on the discrimination of lipids among marine shellfishes.

An essential species in scallop farming is *Patinopecten yessoensis*. Wang et al. [89] showed that 70 °C was the best temperature for hot air drying, and the moisture content of the scallops was less than 20% after 12 h of drying. They investigated the effect of drying on the flavor of scallops by lipidomics. They found that 2,5-dimethyl pyrazine and tetramethyl pyrazine are the characteristic flavor compounds that changed significantly during drying. In addition, taste peptides, such as Arg-Gly and Gly-Gly were found. UPLC-Q Extractive HF-X (ultrahigh-performance liquid chromatography–Q-exactive HF-X orbitrap mass spectrometer) and chemometrics applied, including PCA and PLS-DA (partial least squares discriminant analysis), heatmap analysis, and cluster analysis using MetaboAnalyst 5.0, were the tools applied to analyze the lipid composition of the scallop samples.

A reversed-phase liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (RPLC-Q-TOF-MS/MS) was employed by Cao et al. [209] for the determination of the lipid profiles of three marine fish species, including *Pseudosciaena crocea* (*P. crocea*), *Sebastodes fuscens* (*S. fuscens*), *Cololabis saira* (*C. saira*) and one freshwater fish species *Hypophthalmichthys molitrix* (*H. molitrix*). Comparative analysis was conducted with marine species focusing on the three anatomical parts (head, muscle, and viscera).

A significant abundance of diacylglycerol (DG) was shown in the viscera of four fish species, showing their great potential as functional lipids. Multivariate analysis by PCA and heatmap visualization identified triglycerides (TG) (59:13), diglycerides (DG) (16:1/22:5), and monogalactosyldiacylglycerol (MGDG) (16:0/18:2) as potential biomarkers.

Lu et al. [210] employed iKnife rapid evaporative ionization mass spectrometry (REIMS)-based lipidomics for minced shrimp authentication. A total of 19 fatty acids and 45 phospholipid molecular species were efficiently identified and statistically analyzed by multivariate statistical analysis (PCA) and supervised orthogonal partial least squares discriminant analysis (OPLS-DA). Seven shrimp species were well differentiated.

Zhang et al. [211] studied lipid oxidation and lipid molecule profiling of salted large yellow croaker during storage by high-performance liquid chromatography tandem TripleTOF6600 mass spectrometry (HPLC-TripleTOF6600-MS/MS). The degree of oxidation of protein and lipid was time-dependent, resulting in increased carbonyl content and surface hydrophobicity.

Correlation analysis showed polyunsaturated phosphatidylcholine (PC) to be preferentially oxidized compared to polyunsaturated triacylglycerol.

Table 6 presents examples of lipidomics in fish products.

Table 6. Lipidomics in fish products.

Number	Matrix	Extraction Method	Data Analysis	Results	Reference
1	Fish	Folch method	PCA, cluster correlation analysis	Multivariate principal component analysis showed the lipid composition in shishamo smelt and Japanese sardine.	[212]
2	Salmon	Bligh and Deyer	KEGG functional pathway enrichment analysis	The contents of four lipids lysophosphatidylcholine (LPC) (17:0), LPC (18:0), LPC (22:2), and phosphatidylcholine (PC) (18:4/16:1). were significantly increased from the tenth day.	[195]
3	Tilapia fillets		UPLC-Q-extractive orbitrap mass spectrometry	Lipid composition was affected by raw, steamed, boiled, and roasted tilapia fillets.	[194]
4	Salmon	Rapid evaporative ionization mass spectrometry (REIMS)	Machine learning (ML)-guided REIMS analysis, correlation network analysis	A total of 773 differential expression metabolites (DEMs) were identified and some of them were correlated with lipid oxidation.	[202]
5	Bigeye tuna (*Thunnus obesus*)	iKnife rapid evaporative ionization mass spectrometry (iKnife-REIMS)	Discriminant analysis, support vector machine, neutral network, and machine learning models	Lipid composition was affected during air-frying, roasting, and boiling.	[199]
6	Salmon		Rapid evaporative ionization mass spectrometry (REIMS) combined with an intelligent surgical knife (iKnife). Headspace solid-phase microextraction gas chromatography − mass spectrometry (HS-SPME-GC-MS)-principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA), hierarchical clustering analysis	Salmon samples were subjected to different freeze–thaw cycles. Lipid oxidation was significantly correlated with the number of freeze–thaw cycles.	[201]
7	Octopus	Bligh and Deyer	hydrophilic interaction liquid chromatography-electrospray ionization-mass spectrometry (HILIC–ESI–MS) and tandem mass spectrometry (HILIC–ESI–MS/MS) lipidomics	The polar lipidome of *Octopus* vulgaris has been revealed. The main phospholipids recorded were phosphatidylcholine and phosphatidylethanolamine, while the main sphingolipids identified were phosphonolipid CAEP and ceramides.	[205]

Table 6. *Cont.*

Number	Matrix	Extraction Method	Data Analysis	Results	Reference
8	Surimi		GC- ion mobility spectrometry (IMS), headspace solid-phase microextraction combined with two-dimensional GC–time-of-flight mass spectrometry (GC × GC-TOFMS-orthogonal partial least squares discriminant analysis (OPLS-DA), heat map analysis	Investigated the correlation between flavor-active compounds and 16 differential lipids in composite surimi and discovered that flavor formation was linked with triglyceride degradation and phosphatidylcholine biosynthesis.	[206]
9	Grass carp (*Ctenopharyngodon idella*)	Solid-phase microextraction-gas chromatography–mass spectrometry (SPME-GC–MS	Orthogonal partial least squares discriminant analysis (OPLS-DA)	The lipid oxidation and hydrolysis were promoted by freeze-thaw and heat treatment significantly. Lipid metabolites were analyzed using non-targeted lipidomics and were well distinguished between the different groups.	[213]
10	Crab	Yang et al. (2021) with minor modifications	Ultra-high-performance liquid chromatography highresolution accurate mass spectrometry (HRAM), PCA, partial least squares discriminant analysis (PLS-DA)	Some lipids, such as PE 18:0/20:5, PC 16:0/16:1, PE P-18:0/22:6, and SM 12:1;2O/20:0 could differentiate between *Cancer magister* and *Cancer pagurus*.	[214]

Table 7 presents examples of characteristic markers of proteomic or lipidomic analysis of aquatic and seafood products.

Table 7. Examples of characteristic markers of proteomic or lipidomic analysis of aquatic and seafood products.

Characteristic Markers	Approach	Aquatic and Seafood Products	Aim of the Article	Reference
Peptide biomarkers	Proteomics	Atlantic salmon and rainbow trout	Authentication of Atlantic salmon and rainbow trout	[147]
Protein biomarkers	Proteomics	Grouper fillets	Assessment the deterioration in grouper fillets quality	[196]
Peroxide value	Lipidomic	Sea bass (*Lateolabrax japonicus*)	Lipid quality of seabass by-products	[215]
Aspartic acid	Proteomic	Frozen/thawed and fresh salmon	Discrimination between frozen/thawed and fresh salmon	[119]
15 differentiating lipid compounds	Lipidomic	Marine fish oils from *Lutjanus campechanus*, *Epinephelus lanceolatus*, *Siganus canaliculatus Lates calcarifer* and *Katsuwonus pelamis*	Identification of lipid composition in marine fish oils samples	[12]
Saturated (14:0, 16:0 and 18:0) and unsaturated (20 or 22 carbon atoms) fatty acids and 18:1, 18:2, 18:3 fatty acids	Lipidomic	Wild and farmed salmon	Discrimination between wild and farmed salmon	[216]

8. Combination of NMR-Based Metabolomics and Machine Learning in Fish Aquaculture Quality Evaluation

The combination of NMR-based metabolomics and machine learning in fish aquaculture quality evaluation has also been used with many advantages. NMR plays a prominent role in metabolomics due to its capability to provide a robust chemical overview, and in combination with chemometrics, it becomes a suitable tool for the analysis of complex biological samples, making NMR-based metabolomics a powerful approach for assessing the biochemical responses arising from typical/atypical physiological conditions [217]. NMR metabolic profiling of organic and aqueous sea bass extracts was used to discriminate between wild and cultured sea bass. Sugars, amino acids, dipeptides, and organic acids, as well as metabolites soluble in organic solvents, such as lipids, sterols, and fatty acids, were identified and submitted to principal component analysis [218]. Aru et al. [219] investigated the chemical and microbiological changes in mussels (*M. galloprovincialis*) during storage at 0 °C for 7 days by NMR and OPLS-DA. OPLS-DA models demonstrated significant changes in the concentration of amino acids, organic acids, and osmolytes, which reflected the presence of microbial growth under cold storage conditions. Locci et al. investigated the molecular components of the aqueous extract of samples of bottarga, that is, salted and dried mullet (*Mugil cephalus*) roe, manufactured in Sardinia (Italy) from mullets of known and unknown geographical provenience, by NMR combined with multivariate data analysis (MVA). They indicated that samples tend to cluster according to their geographical origin and also on the basis of storage and manufacturing procedures [220]. An NMR metabolomics approach coupled with OPLS-DA was applied to analyze the changes in the metabolic profile of the bivalve *M. galloprovincialis* during storage at 0 °C and 4 °C for 10 and 6 days, which revealed a clear distinction between fresh samples and those stored at 0 °C and 4 °C [221]. The work of Ciampa et al. proposed a method to calculate trimethylamine content and K-index value, which are biomarkers of freshness in different fish species based on HR ^{1}H-NMR, as an alternative method capable of evaluating both indices simultaneously in aqueous fish extracts [15].

9. Future Perspectives

In the future, biomarker development as well as integration of the aforementioned omic technologies and their application to the overall quality management system are expected to have a major impact on the food safety and management systems of the industry. Determining the precise content of specific biomarkers in aquatic products remains a challenging task. Taking everything into consideration, in the future, technology transfer and scale-up omic methods should be tested in order to successfully incorporate that knowledge into the food industry and responsible for food regulatory agencies. New omics technologies and in the field of aquatic products to optimize their safety and authenticity could be the peptidomics and flavoromics. Advances in mass spectrometry technology and the discovery of bioactive peptides from food sources have led peptidomics to rapidly develop in the field of food science. The flavoromics approach seeks to reveal the mechanism behind the formation of flavors in aquatic products and to evaluate their nutrients [222].

10. Conclusions

The quality and safety of seafood and aquatic products is of particular concern to the entire food chain, since these foods are extremely popular and in high demand by consumers. Omic research has developed many important tools through which quality factors are controlled. Omics-integration approaches, such as metabolomics, proteomics, lipidomics, volatolomics, and metagenomics, provide a wealth of data that, when combined with chemometrics and predictive models, can ensure the quality control of aquatic products regarding microbial growth, freshness, lipid oxidation, authentication, production method, storage condition, geographical origin and classification issues, chemical contaminants, and veterinary drug residues. Omic approaches are undoubtedly a useful

and desirable tool that can contribute to the analysis of these highly vulnerable foods with excellent nutritional values, such as seafood and aquatic products.

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