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Review

Mycotoxins in Seafood: Occurrence, Recent Development of Analytical Techniques and Future Challenges

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Abstract: The co-occurrence of mycotoxigenic fungi and mycotoxins in aquatic food commodities has recently become a source of severe worldwide food insecurity since these toxicants may damage human health. The consumption of aquatic food itself represents a relatively novel and non-negligible source of mycotoxins. Mycotoxins in seafood lead to important human genotoxins, carcinogens, and immunosuppressors. Consequently, it is crucial to quantify and characterize these contaminants in aquatic food products subject to extensive consumption and develop new regulations. The present paper provides an overview of recent advancements in liquid chromatography and mass spectrometry and the coupling of these techniques for identifying and characterizing mycotoxins in various fresh, comestible, and treated marine products. The disposable data display that a multiplicity of fungal species and further mycotoxins have been detected in seafood, comprising aflatoxins, ochratoxins, fumonisins, deoxynivalenol, zearalenone, and trichothecenes. In addition, a wider and up-to-date overview of global occurrence surveys of mycotoxin occurrence in seafood in 2017–2022 is explored. In this regard, the predominant occurrence of enniatins has been documented in seafood products. Likewise, special attention has been given to current EU seafood legal and existing national regulations of mycotoxins in seafood. In this way, rigorous national and international guidelines are needed for palpable and effective measures in the future. Nevertheless, controlling mycotoxins in aquatic foods is an ambitious aim for scientists and industry stakeholders to ensure sustainable global food safety.

Keywords: mycotoxigenic fungi; mycotoxins; seafood; occurrence; analytical strategy

Citation: Smaoui, S.; D'Amore, T.; Agriopoulou, S.; Mousavi Khaneghah, A. Mycotoxins in Seafood: Occurrence, Recent Development of Analytical Techniques and Future Challenges. *Separations* **2023**, *10*, 217. https://doi.org/10.3390/separations10030217

Academic Editor: Paraskevas D. Tzanavaras

Received: 29 January 2023
Revised: 15 March 2023
Accepted: 17 March 2023
Published: 20 March 2023

1. Introduction

The consumption of seafood products is widespread, particularly in coastal regions characterized by a hot and humid climate [1,2]. Fresh fish and fish products are spoilable and are handled using several techniques to enhance their shelf life. In this line, controlling water activity (a_w) is these techniques' most important processing phase [2–5]. For efficient preservation, several methods are employed, viz., freezing, chilling, canning, salting, drying, and smoking [3,6,7]. Among them, drying and salting are the most popular common methods. For instance, in Zhanjiang, a province in southern China, the yearly fresh fish production is higher than 100,000 kg, and dehydrated salting of fish is the conventional tool to preserve seafood [2,8,9]. Through the drying and curing process, exceptional desired flavors are produced. Nevertheless, seafood drying is conducive to food safety concerns

since the process needs to be better governed. In several African and Asian countries, marine products are directly dried in the open air [3,10–14]. This approach could be more effective, since it only partially mitigates microbial load. Numerous investigations have reported that dried fish quality deterioration is caused by fungal growth [15–19]. Consequently, fungal contamination influences the quality of fish through texture and flavor degradation, nutrient loss, and special food safety concerns, and, therefore, causes massive financial losses to the industry. To provide details for hazard valuation of dehydrated marine product security, several investigations were conducted to study fungal diversity. According to several reports, fungal genera infecting dehydrated fish are *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., and *Fusarium* sp. [20–22]. For example, Nigerian smoked fish was found to be contaminated with *Aspergillus flavus, Fusarium oxysporum, Ceotrichium albidium, Rhizopus* spp., *Penicillium* spp., and *Trichoderma* spp. [23–26]. In Kenya, *Aspergillus niger* and *Rhizopus* spp. at 17.57 and 29.73%, respectively, were the main fungal species detected from dehydrated fish [27,28]. These fungal species are susceptible to producing mycotoxins in seafood, and the extremely frequent ones are: aflatoxins (AFs); *Fusarium* mycotoxins, i.e., trichothecenes (deoxynivalenol (DON); T-2 toxin, HT-2 toxin); zearalenone (ZEN) and fumonisins (FBs); and ochratoxins (OTs) [29–31]. According to their chemical structures, mycotoxins are stable, and their elimination from the food chain is complicated [32,33]. Generally, food processing reduces mycotoxin levels somewhat but does not remove them completely. In this regard, though, very high temperatures, extrusion, and roasting present potential for lowering mycotoxin levels. Extrusion processing at temperatures greater than 150 °C is necessary to reduce ZEA quantitatively; on the contrary, this process only reduces Afs modestly, and very variable reduction rates have been reported for DON and FUMs. The greatest reductions of FUM occur at extrusion temperatures of 160 °C or higher [34–36].

Huang et al., 2011, detected AFB1 in the muscle and hepatopancreas of gibel carp (*Carassius auratus gibelio*) at 2.4 and 11.8 µg/kg, respectively [32]. From a Nigerian market, Fagbohun and Lawal (2015) identified 2.73–4.03 µg/kg AFB1 and 2.01–3.53 µg/kg AFG1 residues in 50 smoked dried fish samples [25]. Wozny et al., 2013, reported an amount of ZEA equal to 7.1 µg/kg in ovary rainbow trout [33,37]. Additionally, Gonkowski et al., 2018, analyzed sun-dried fish in Zambia and detected ZEN in all samples [38]. Currently, the emerging *Fusarium* mycotoxins beauvericin (BEA) and enniatins (ENNs), with ENNB as the most predominant isoform, have been detected in European marine aquafeeds, with enniantin B (ENNB) displaying about 90% prevalence in all tested feed samples [39,40]. ENNs were perceived in the fillet of commercially farmed European sea bass (*Dicentrarchus labrax*) and gilthead sea bream, while BEA was not detected in these fish [41]. Likewise, the predominant mycotoxins in Norwegian-farmed Atlantic salmon fillets were ENNs [42]. Additionally, several enniatins (ENNs; ENA1, ENB, and ENB1) were reported in seabream, seabass, tilapia, and panga tissues from commercialized aquaculture fish by Tolosa et al. [43]. Zhao et al. [44] reported that the presence of mycotoxins in products of animal origin is directly affected by mycotoxin contamination in feed, as they can be retained in organs and edible tissues after metabolism and can also be excreted in some by-products. A similar discovery was made in a study in which the authors detected and identified 70 representative compounds in fish feed and fish fillets, including antibiotics, pesticides, and mycotoxins [45]. The occurrence of ENNs instead of other mycotoxins allows us to conclude that these mycotoxins could be present in the edible tissues of fish consuming these contaminated feeds.

Before advanced examination, mycotoxin detection, quantification, and differentiation are crucial. Sampling, sample pretreatment, and preparation methods must be reproducible and accurate to ensure high selectivity and reliability, excluding matrix interferents and shifting, since an analytical technique will be employed [39,40]. The extraction techniques most described in the literature for sample pretreatment of mycotoxins are the standard extraction approaches: LLE and SPE. Moreover, due to sustainability and green chemistry, SPME has become very popular [41]. At the same time, amongst the practically employed extractive phases is IAC [42], and LC approaches are the most used. These chromatographic

systems have been combined with several detectors, such as UV and fluorescence, and mass spectrometers to extend the applicability domain and number of mycotoxins analyzed in a single working session, as well as improve analytical performances. For mycotoxin determination, several analytical methods, including TLC [46,47], a pioneering technique for detecting these compounds, ELISA [48], GC-ECD [49,50], GC-MS [51,52], UPLC/FLD, and UPLC-PDA [53,54] have been established. Undoubtedly, LC-MS/MS performs a crucial role in mycotoxin analysis due to the intrinsic high sensitivity and selectivity of mass spectrometer detectors [55–59]. Therefore, these analytical techniques are widely applied to analyze seafood mycotoxins and other trace contaminants.

Mycotoxins are a group of toxic secondary metabolites produced by fungi that can contaminate various food products including seafood [60]. These toxins pose a threat to human health, as they can cause a range of health issues, including allergic reactions [61–63]. Allergic reactions to seafood are a common occurrence, and mycotoxins can exacerbate these reactions [64,65]. Mycotoxins can increase the allergenicity potential of seafood products by disrupting the integrity of the gastrointestinal tract. The gastrointestinal tract acts as a barrier, preventing large molecules from entering the bloodstream. When mycotoxins are present, they can disrupt this barrier, making it more permeable to allergens. This can result in an increase in allergic reactions to seafood products that are already known to be allergenic, such as shellfish and fish [66–69]. Moreover, some mycotoxins have been shown to directly induce allergic reactions by stimulating the immune system. For example, ochratoxin A, a common mycotoxin found in seafood, has been shown to increase the production of IgE antibodies, which are responsible for many allergic reactions [70–73]. The presence of mycotoxins in seafood products can also result in a higher allergenicity potential through cross-reactivity. Cross-reactivity occurs when the immune system confuses similar proteins between different food sources, leading to an allergic reaction. Mycotoxins can modify the proteins in seafood products, resulting in similar protein structures to those found in other food sources. This can lead to cross-reactivity and increased allergenicity potential in affected seafood products [74–78]. In conclusion, mycotoxins can increase the allergenicity potential of seafood products in several ways, including disrupting the gastrointestinal barrier, direct stimulation of the immune system, and cross-reactivity. This is a significant concern for public health, and measures to reduce the levels of mycotoxins in seafood products should be taken to minimize the risk of allergic reactions [79–83]. On the other hand, the consumption of seafood contaminated with mycotoxins has been associated with many human health effects (acute and chronic toxicity), varying from gastrointestinal symptomatology to immune deficiency and even carcinogenicity, reproductive disorders, organ failure, hepatotoxicity, genotoxicity, neurotoxicity, pulmonary edema, convulsions, coma, and death [60,84–86]. As reported by IARC (2012), AFs (AFB1, AFB2, AFG1, and AFG2) have been categorized in group 1 as human carcinogens [60,87]. For example, studies have established that meat fish aquaculture contaminated with AFs can provoke depressive and neurological disorders, testicular toxicity, general pain, estrogenic effects, gastrointestinal disorders, and other damaging health effects [88]. Similarly, OTA ingestion has been connected to many diseases, such as urothelial urinary tract tumors and renal intumescences [89,90]. Acute and chronic absorption of DON, T-2 toxin, HT2, and ZEA (produced by *Fusarium*) can cause many toxic effects comprising hemorrhaging, emesis, diarrhea, immunosuppression, leucopenia, reduced reproductive capacity, bone marrow injury, and radiomimetic injury of tissues [91–93].

The authorities set the maximum levels for these mycotoxins worldwide for these reasons. For AFB1, these limits are 2 and 20 μg/kg for EU and China, respectively. For EU and China, the OTA limits are 5 μg/kg, 1250 μg/kg (EU), 1000 μg/kg (China) for DON, and 100 μg/kg (EU) 60 μg/kg (China) for ZEN [94]. The EFSA has set the TDI for humans to 0.1 μg/kg body weight for the sum of T-2 and HT2 toxins [95].

Mycotoxin concentrations in fish and fish products must be regularly monitored to guarantee food safety. An approach to the occurrence and analytical determinations of

mycotoxin-contaminated seafood products is outlined in this review. In addition, we investigated the diversity of contaminated fungi species in seafood products.

2. Regulation of Mycotoxins in Seafood

The demand for seafood from aquaculture and capture fishery production have been increasing in recent years; in the same manner, the search for alternative feeding purposes for marine animals, including plants, cereals, seaweed, and vegetable oils, is very active. These changes in marine feed ingredients are associated with new potential risks, such as introducing contaminants not previously associated with marine animals, i.e., microorganisms, fungi, mycotoxins, pesticides, tropane, pyrrolizidine alkaloids, and even cannabinoids [96,97]. Moreover, a route of contamination with these unusual contaminants is the phases of storage and processing of seafood. This transfer of unwanted contaminants from one matrix to another is known as a carry-over effect. In this specific regard, the phrase "feed-to-fillet transfer" has been used to describe this event [98]. Scientific literature has increasingly described this potential food safety and welfare issue. Salmon, gilt-head bream, and shrimp are only some of the matrices in which several mycotoxins were detected. In many studies, the authors also attempted to establish the transfer factor from feed matrices to marine animals [99]. At the same time, much research was conducted describing the severe adverse effects of mycotoxin contamination of aquatic organisms (mycotoxicosis). The decreased body weight of fishes, mollusks, and crustaceans, growth impairment, kidney and liver degenerative lesions, and high rates of diseases and mortality are the main harmful effects reported and endpoints studied. This may result in a decrease in fishery productivity [88,100–102]. However, the available information on the concentrations of several mycotoxins in seafood must be more cohesive and harmonized. The EFSA identified uncertainties and data gaps in its reports and hazards for people and animal wellness linked to mycotoxins in food and feed. As a result of the absence of occurrence or toxicological data for marine products, EFSA reports underlined that the risk was not determined. According to the EFSA, CONTAM has derived the toxicological parameter (NOAEL = 13 μg T-2/kg body weight/day) only for trichothecenes in fish. Similarly, a LOAEL = 10 mg/kg was stated for fumonisins according to morbidity modifications in the heart, kidney, pancreas, and brain. For other mycotoxins, the CONTAM panel had insufficient occurrence data available in seafood matrices; subsequently, no detection and hazard assessments were carried out [103–105].

From these new findings and technological evolutions, a new scenario emerges. The new concerns along all marine food chains require improving and harmonizing regulations worldwide. The current legislation has no established maximum limits or monitoring programs for mycotoxins in seafood. On the contrary, practically all organizations and countries have set regulations or guidelines for maximum concentrations of mycotoxins in vegetable food matrices and animal feed. Establishing new regulations in food law is a very complex activity that requires the efforts of several actors, such as researchers, risk assessors, experts, industries, and regulatory agencies. Moreover, apart from the general precautionary principle where RMMs are adopted when there is reasonable doubt of a harmful effect on humans and the environment by an agent, economic factors also play a big role. However, the principle "No safety, no market" is generally applied in almost all worldwide regulations. Hazard identification and characterization, toxicological data, and exposure assessment are fundamental for risk characterization and assessment. After this, if it is necessary to mitigate the risk, specific RMMs are proposed, such as a proposal of law, the set of maximum residual limits, techniques, and practices in place to reduce risks. All the decisions follow accessible scientific knowledge, expert judgment, and the weight of evidence (Figure 1).

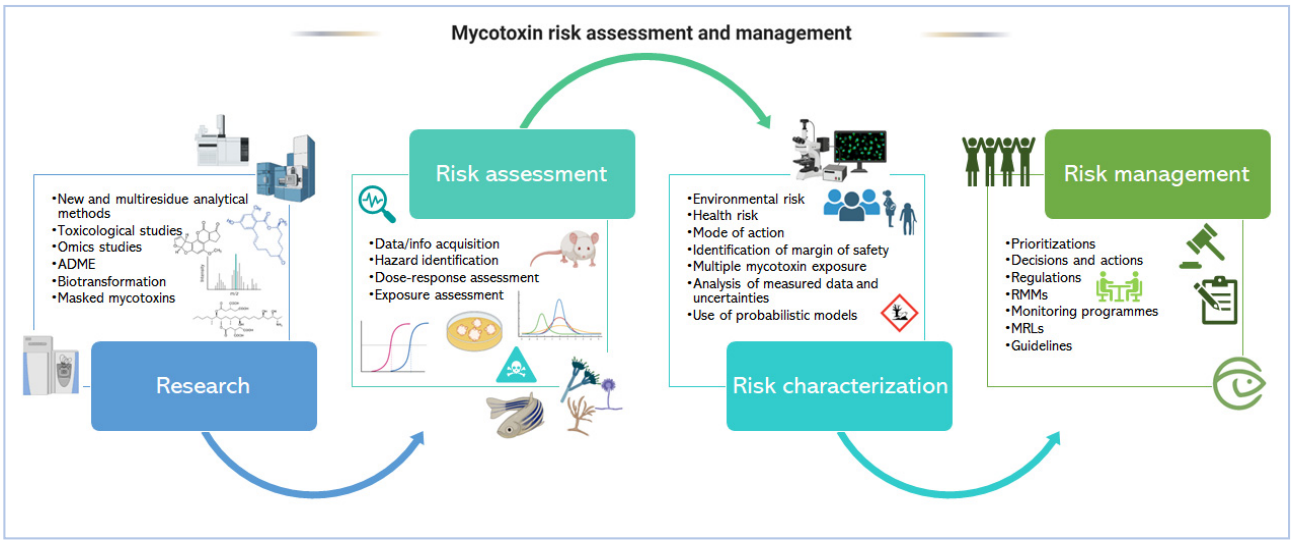

Figure 1. Risk assessment and management of mycotoxins in seafood: The possible development framework of the new regulation.

In particular, mycotoxin management in all matrices, including seafood, is a major issue for all governments. For example, it is included in the "Farm to Fork strategy" and the EU Green Deal [106,107]. The fact that mycotoxins represent a main food safety hazard is further confirmed by consulting and analyzing the notifications on the RASFF portal. This system was created by the European Commission to ensure the flow of information and as a useful mechanism to inform European citizens about food hazards as defined by article 50 of Regulation EU No. 178/2002 [108,109]. This analysis considered the last three years' notifications (from January 2020 to October 2022). In this time window, the notifications reported from Member States in all hazard categories, i.e., 26 categories, all attributable to four primary categories of food safety hazards, (1) chemical, (2) physical, (3) biological, and (4) allergenic, numbered 12,079. Among them, 1295 notifications are related to mycotoxin contaminations of food and feed, representing 11% of all notifications; additionally, in 95% of those cases, the risk decision was labeled as serious. Moreover, the authors believe these data should be accounted for more because the official controls were concentrated only on a few matrices, i.e., cereals, nuts, seeds, fruit and vegetables, spices, and herbs.

Undoubtedly, data gaps and several uncertainties for my-seafood hazard exist. Risk characterization and assessment require new targeted translational studies focusing on environmental fates and trophic transfer of mycotoxins in the aquatic compartment. Similarly, the toxic significations, co-occurrence of mycotoxins, and probable risks for human health and the environment must be clarified [96].

3. Occurrence of Mycotoxins in Seafood

The consumption of mycotoxin-contaminated feed by aquaculture fish has adverse effects on their health, with important risks [110,111]. In addition to the adverse effect that the consumption of contaminated fish feed can bring, the harmful effect that will be caused to the health of everyone who consumes these foods is also expected [102]. Cereal-derived aquatic feeds are severely affected by mycotoxins, as cereals are a major category of products contaminated by mycotoxins [112,113]. Aside from cereals, legumes such as soybean and peanut are also used for aquatic feeds.

Feed can be contaminated both at the pre-harvest stage and later. Conditions related to weather changes and extreme weather events significantly affect mycotoxin contamination. High temperature and humidity in the storage period have contributed to increased contamination [114]. In particular, feed handled in developing countries was more likely to be contaminated by mycotoxigenic fungi and mycotoxins due to tropical climates, poor

agricultural practices and technologies, and poor storage conditions. Since feed represents one of the most traded products, mycotoxin-contaminated feed may occur anywhere in the world [115]. In these regards, *Aspergillus flavus* and *Aspergillus parasiticus* have been detected worldwide in the soil and the air, with optimal growing conditions at temperatures between 22 and 35 °C and a_w between 0.95 and 0.98. These findings are associated with some considerations. An efficient method to reduce the production of AFs is maintaining the temperature in the storage area below 15 °C and consequently lowering the a_w for the production of mycotoxins to 0.934. These mitigation strategies cannot be applied to other species; *Penicillium verrucosum*, responsible for the production of OTAs, has a wide growing temperature range (0–31 °C) and, in consequence, OTA is a ubiquitous mycotoxin [116]. Similar worldwide diffusion is reported for FUMs, mainly produced by *F. verticillioides* and *F. proliferatum* in favorable high-temperature and humid climates [117].

The increased demand for seafood is because it is an excellent and healthy dietary option [118,119]. About 17% of global protein consumption comes from seafood, indicating that it is a significant nutritional source [120]. The FAO recently announced that global seafood production reached 179 million tons in 2018. Seafood production has quadrupled over the last half century as global consumption has increased. In order to meet the ever-growing demand, seafood production has changed, with aquaculture contributing more and more. According to FAO's "The state of world fisheries", production from aquaculture amounts to 80 million metric tons, accounting for 46% of global fish production [121]. In RASFF reports from 2004 to 2018, although mycotoxins have the largest proportion of notifications (9522 out of a total of 42,181), there was only one notification for fish and fish products, indicating that these food categories do not belong to foods that pose serious "mycotoxin risks". Moreover, until now, the importance of mycotoxins in aquaculture has yet to emerge compared to that associated with other animals [111].

Of the few available studies that have been performed on seafood and the occurrence of mycotoxins, predominant mycotoxins were found to be AFs, ENNs, DON, ZEA, and OTA [33,42,84,111,122–124].

ENN analyses on various fish species, including *Dicentrarchus labrax*, *Sparus aurata*, *Salmo salar*, and *Onchorhynchus mykiss*, found contamination at levels (1.3–10^3 ppb) [58]. Tolosa et al., 2020, explored mycotoxin levels in *Salmo salar* and characterized 40 different forms of mycotoxins, mostly ENNs [42]. Tolosa et al., 2014, assessed the occurrence of ENNs in *Dicentrarchus labrax* and *Sparus aurata,* edible fishes [123]. The findings indicate that the greatest levels of ENNs were found in the liver and muscles. The ENA1, ENB, and ENB1 levels were 1.76.9, 1.344.6, and 1.431.5 ppb, respectively. In *Sparus aurata* muscles, they were 2.17.5, 1.3–21.6, and 7.1 19 ppb, respectively [123]. Tolosa et al., 2013, studied the BEA and ENNs in farmed *Dicentrarchus labrax*, *Sparus aurata*, *Oreochromis niloticus*, *Pangasius bocourti,* and *Mackerel hake* [43]. These authors confirmed that ENB (1.26 ppb) was the most common mycotoxin. Berhnhoft et al., 2017, reported that the OTA occurrence in post-smolt Atlantic salmon (*Salmo salar*) was mainly distributed in the liver and kidneys [125]. The occurrence of mycotoxins in Serbian *Cyprinus carpio* revealed that mycotoxin levels in muscle tissue samples varied from 0.4 ppb for AFs to 30 ppb for DON, which was < LOQ [2,126]. El-Sayed and Khalil (2009) reported that *Dicentrarchus labrax,* a fish farmed in Al-Behera, Egypt, was contaminated with AFB1 (0.30 mg/kg) [127]. Orony et al., 2016, found that 17% of fish (*Lates niloticus*, *Oreochromis niloticus*, and *Rastrineobola argentea*) muscle tissues contained DON [119]. Woźny et al., 2013, assessed ZEN in feed and *Oncorhynchus mykiss* muscle purchased from commercial fish farms in northeastern Poland. The corresponding concentration was equal to 8.18 ppb [33].

Generally, there are three plausible paths whereby mycotoxins contaminate seafood products: (i) microbes, such as bacteria, molds, and yeasts and/or spores, contaminate the seafood surface during the drying operation, with consequent fungal development and mycotoxin synthesis; (ii) inappropriate preservation, e.g., contact with elevated temperatures/humidity; (iii) secondary transfer, e.g., consumers or vendors contaminate the seafood products when they handle them at the market.

Table 1 shows the occurrence of mycotoxins in seafood in the last 5 years (2017–2022).

Table 1. Occurrence of mycotoxins in seafood in last 5 years (2017–2022).

Seafood Species	Mycotoxins	Fish Tissue	Mycotoxins µg/kg	Analytical Method	Reference
Sparus aurata Dicentrarchus labrax	OTA	Muscle	LOD-0.28	HPLC-FLD	[128]
Salmo salar	Forty mycotoxins	Fish fillets	NA	LC-Q-TOF-MS	[42]
	ENNA1		1.7–6.9		
Dicentrarchus labrax	END		1.3–12.8		
	ENNB1	Fish fillets	1.4–31.5	LC-MS/MS	[58]
	ENNA1		2.1–7.5		
Sparus aurata	END		1.3–21.6		
	ENNB1		7.1–19.0		
	ENNA1		22–29		
Salmo salar	ENNB		50–103		
	ENNB1		56–94		
	ENNA1		-		
Oncorhynchus mykiss	END		3.6		
	ENNB1		2.9		
Round fish	AFB1	Round fish muscle	0.15–5.70	HPLC-FLD	[129]
Oreochromis niloticus	AFB1	Fish muscle	0.09–0.37	HPLC-FLD	[124]

4. Recent Analysis Approaches

Mycotoxins were pretreated and extracted from food matrices by several approaches. Solvent extractions protocols are usually combined with other treatments, especially for complex matrices. In particular, several "QuEChERS" extraction or solid-phase extraction protocols coupled with homogenization and centrifugation have been described. Using QuEChERS for extraction has been found to be a helpful tool to be applied in different seafood matrices. For instance, Tolosa et al. [130] used a QuEChERS extraction procedure with an acidified MeCN/H_2O solvent mixture to evaluate AFs, FBs, ENNs, BEA, FUS-X, and OTA. Similarly, Wang et al. used a modified QuEChERS protocol with methanol/ethanol/isopropanol (7:2:1, $v/v/v$) as the solvent combined with high-efficiency dSPE using graphene oxide (GO) as the sorbent and freezing for the removal of pigments and lipids for extracting mycotoxins from shellfish samples [131]. Specific clean-up methods based on immunoaffinity columns (IACs) or combinations with another clean-up technique were used by Lattanzio et al. for the detection of Afs and OTA. However, QuEChERS has the highest applicability among these cleanup techniques, so it is particularly useful for multi-mycotoxin determination.

Additional extraction steps such as DLLME, SPE, or portioning may be necessary for some particular rich or interfered matrices.

Regarding the mycotoxin in the seafood extraction step, some modern microextraction systems, such as DLLME, were recently established. In the study of Tolosa et al., 2019), the existence of 15 mycotoxins in processed fish products was evaluated [130]. The extraction of several mycotoxins (AFs, FUMs, ENNs, BEA, FUS-X, and OTA) from fish matrices was performed using a modified QuEChERS method. A supplementary step with C18 sorbent was added to improve recoveries and significantly reduce the matrix effect. After applying UAE, DLLME was employed to purify mycotoxins in the sea bass side stream (head, skin, bones, and viscera) extracts before determination [130]. These authors used water and these conditions: 20 kHz, 100 W, 30 min, and 30 °C. After applying UAE conditions, AFBs, OTA, ZEA, and ENNs were detected under the LODs in sea bass side stream at 0.58–0.89, 0.55–1.34, and 0.36–1.51 µg/kg, respectively. Deng et al., 2021, analyzed mycotoxin in seven dried fish species (*Hemibarbus maculatus*, *Pseudosciaena crocea*, *Lutjanus erythopterus*, *Thunnus thynnus*, *Scomberomorus niphonius*, *Eleutheronema tetradactylum*, *Trichiurus lepturus*) [132]. These were sampled from seafood markets in Zhanjiang, China [2]. For extraction, these authors utilized UAE at (60 min, 20 °C) and an acetonitrile/water mixture (85/15, v/v) as an extraction solvent to analyze 40 dried seafood products [2]. Sun et al., 2015, employed a

UAE protocol followed by LC-MS/MS separation and detection to simultaneously determine mycotoxins in fresh fish and dried seafood [95]. The extraction method was boosted to increase proper recoveries. In this vein, for mycotoxin extraction, the optimum process comprised UAE with acetonitrile/water/acetic acid (79/20/1, *v/v/v*) at 40 °C for 30 min. N-hexane and Oasis HLB cartridges were used for decreasing and cleanup, respectively. Regarding recoveries, the mean values ranged between 76 and 111%, 72 and 116%, and 72 and 120% for muscle, entrails, and dried fish products, respectively. These values are between 70 and 120% according to the European Union SANCO/12495/2011 guidelines and the Brazilian Manual of Analytical Quality Assurance. In addition, the method was affirmed by assessing the linearity ($R^2 \geq 0.9989$), sensitivity (LODs ≤ 2 µg/kg, LOQs ≤ 3 µg/kg), and precision ($\leq$18.3%) in all marine product samples. After the implementation of the settled method in 27 seafood samples, AFB2, OTA, and ZEN were detected. A total of four samples were contaminated with OTA, ranging between 0.5 and 1.9 µg/kg. AFB2 at 1.2 292 µg/kg was planted in the carp sample. Regarding ZEN, this mycotoxin demonstrated a prevalence of 29.6% and a concentration = of 317.3 µg/kg, which was identified in dried samples [95]. The efficiency of mycotoxin extraction via UAE in animal-derived food was greatly upgraded by optimizing parameters such as solvent mixtures, temperature ramp, and extraction time [133].

Through DLLME and HPLC-MS/MS, Tolosa et al., 2016, detected 15 mycotoxins in Spanish fish plasma samples [57]. Considering that the kind of extractant was the most crucial with regard to DLLME efficiency, these authors tested two distinct solvent matrixes: $CHCl_3$ and EtOAc. In this study, EtOAc was selected as the optimal extraction solvent. For instance, the recoveries of AFB1 increased from 90 to 120% when using EtOAc. On the other hand, recoveries of other mycotoxins were kept constant, excluding *Fusarium* mycotoxins, which were lessened once $CHCl_3$ was employed. DLLME has become popular since it is considered green, embracing the main principle of green analytical chemistry. Moreover, this method benefits traditional techniques due to its simplicity, speed, and low cost [134]. One of the main advantages of DLLME is that the contact surface of extraction solvent to aqueous sample is particularly high; in this way, the extraction phase becomes brief compared to other approaches, as the equilibrium state is steadily reached. In DLLME, numerous agents can impact extraction effectiveness, comprising the dispersive solvents and the kind of extraction, in addition to salt added for the salting-out process [135].

Carballo et al., 2018, defined mycotoxin occurrence in five food groups: cereals, legumes, vegetables, fish, and meat [55]. For extraction, these authors engaged QuEChERS linked with d-SPE [136]. In marine products, the occurrence was 9% below the greatest limit denoted by the EU [136]. At 1.19 µg/kg, DON was the most-detected mycotoxin, with an incidence of 19%. In addition, ENNA and DAS were detected in fish products at 0.89 and 7.0 µg/kg, respectively. Principal component analysis was developed to assess plausible associations between different studied mycotoxins in fish samples. In this regard, 3ADON, NEO, ENNA, DAS, and 15ADON displayed good correlation. It should be noted that this correlation can be conducive to an increase in toxic activity. For instance, a dual blend of (3ADON-15ADON) and (ENB-DON) at 24 h demonstrated a boost of cytotoxic effect [137,138]. Via ELISA-based methods using AFB1, OTA, and FB1 standards and commercially available detection kits, Huong et al., 2016, reported a lower level of OTA in dried shrimp samples (4850 ng/kg) and Vietnamese fish products (1770–2720 ng/kg) [139]. Similarly, Tsafack Takadong et al., 2020, assessed the presence of AF AFB1 in four fish species (kanga, tilapia, catfish, and carp) in two Cameroonian localities by using immunological assays [140]. As reported by these authors, the most contaminated species was catfish, and the least contaminated one was tilapia. Only catfish presented a level of AFs and AFB1 higher than the recommended attention level set by the FDA for these toxins (20 µg/kg). The authors also monitored mycotoxin levels in feed and concluded that the carry-over effect was responsible for fish contamination.

According to Tolosa et al., 2019, the identification and quantification of extracted mycotoxins were performed using liquid LC-MS/MS-LIT. This investigation revealed ENN

B and FUS-X in the gula substitute [130]. According to these authors, this fact might be caused by using contaminated ingredients or products in development due to unsuitable elaboration or the preservation status needing to be improved.

Saad et al., 2020, collected 90 fish sample products from a distinct Egyptian market. These products were smoked herring, canned sardines, and frozen fish fillets. By employing HPLC, aflatoxins and ochratoxin A were detected [141]. Overall, the samples were infected by AFB1. This mycotoxin was detected in 20, 30, and 43.33% of frozen fish fillets, smoked herring, and canned sardine samples, respectively. With regard to aflatoxin B2 and AFG1, 20% of the studied samples were tainted. Regarding AFG2, 11.1% of the fish product samples were contaminated, and the incidences in the investigated samples were 13.33% (smoked herring), 13.33% (canned sardine), and 6.67% (frozen fish fillets). The authors stated that smoked herring samples had 51.63 μg/kg of AFB1, 37.29 μg/kg of AB2, 25.06 μg/kg of AFG1 μg/kg, and 16.22 μg/kg of AFG2. Canned sardine samples were contaminated by AFB1, AB2, AFG1, and AFG2 at 33.14, 20.81, 14.42, and 11.29 μg/kg, respectively. Globally, smoked herring had the highest levels of mycotoxins, succeeded by canned sardine and frozen fish fillets, and AFB1 was the principal detected mycotoxin.

By LC-MS/MS, Deng et al., 2020, quantified four mycotoxins, namely AFB1, DON, OTA, and T-2, in 40 Chinese dried seafood (mussel, shrimp, and fish) products [132]. LOD and LOQ ranged between 0.1 and 2.0 μg/kg and 0.3 and 5.0 μg/kg, respectively. Regarding recoveries, intra- RSDs and inter-RSDs were arrayed between 72 and 98%, 2.8 and 10.6%, and 5.5 and 15.4%, respectively. With incidences of 30.8, 33.3, and 17.2%, AFB1, OTA, and T-2 were the most common mycotoxins in the investigated dried seafood samples. Regarding mycotoxin levels, AFB1, OTA, and T-2 averaged 0.58–0.87, 0.36–1.51, and 0.55–1.34 μg/kg. Later, these authors, Deng et al., 2021, investigated the diversity, occurrence frequency of total tainted fungal species, and chemical mycotoxin analysis of Chinese dehydrated fish products obtained from Zhanjiang province [2]. Analysis using LC-MS/MS found that AFB1 was detected at 0.03 to 3.52 μg/kg, T-2 at 0.21 to 1.53 μg/kg, OTA at 0.03–2.21, and DON at 0.71 μg/kg [2].

5. Fungi Contamination of Marine Products

Deng et al., 2021, examined the diversity of fungal species of fish products obtained from China [2]. Three major genera were detected in dehydrated fish samples: *Fusarium* sp., *Penicillium* sp., and *Aspergillus* sp. at 80.4, 70.7, and 63.89%, respectively. It should be noted that *A. flavus* and *Fusarium* sp. contaminated sample levels ranged between 1.10×10^3 and 2.40×10^4 CFU/g and 1.09×10^2 and 2.11×10^4 CFU/g, respectively; nonetheless, the occurrence was comparatively elevated. According to some reports, general fungal genera adulterating dried fish are *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., and *Fusarium* sp. [20]. According to Ayeloja et al.'s (2018) study, *A. flavus, F. oxysporum, C. albidium, Penicillium* sp., and *Trichoderma* sp. were isolated from smoked fish sold in Ibadan Oyo State, Nigeria [23]. In another study conducted by Fagbohun and Lawal (2015), with a charge load between 4.7×10^2 to 9.1×10^4 CFU/g, 11 different fungal species attributed to six genera were linked with smoked dried fish samples isolated from Nigerian trade sites [25]. Among them, *A. flavus* and *Penicillium* spp. had the higher amounts of occurrence, and AFB1 (2.731–4.031 μg/kg) and AFG1 (2.015–3.528 μg/kg) were detected in Cat and Sole fish (*Gymnallabes typhus and Cynoglossus browni*) and West African Shad (*Ilisha africana*) samples. On the other hand, Nyamwaka et al., 2017, noted contamination frequencies of *A. niger*, *A. flavus*, and *A. fumigatus* at 17.6, 9.5, and 10.83%, respectively [28]. Notably, the genus *Aspergillus* was pointed out to produce spores and mycelium, expanding the danger of fungal production in dried fish [117]. From farmed fish such as *Oreochromis niloticus*, *Clarias gariepinus*, *Tilapia zilli*, *Nematalosa erebi*, and *Chelon ramada* species, Hashem (2011) confirmed the presence of *Aspergillus* species, *P. chrysogenum*, and *Trichoderma viride* [142]. Another study assessing Cameronese farmed fish *Heterotis niloticus*, *Oreochromis niloticus*, *Clarias gariepinus*, and *Cyprinus carpio* for total AFs and AFB1 evidenced that all fish tissues were infected [140]. Osibona et al., 2018, studied the occurrence of storage fungi and their

respective mycotoxins in stored smoked dried fishes [143]. These authors reported that the stored samples were associated with *A. flavus*, *A. fumigatus*, *A. niger*, *A. wentii*, and *Penicillium* sp. Four species of *Aspergillus* and one *Penicillium* species were isolated and identified from two of three storage conditions (bamboo basket and iron basket) from the third week of storage. Moreover, AFB1 and OTA were found in the samples of the two storage types (the above-stated containers). Hissein et al., 2019, screened the presence of fungi and AFs in dried and smoked *Clarias* sp. and *Oreochromis* sp from Lake Fitri–Chad, revealing contamination with *A. niger* and *A. fumigatus* (at 66%) [144]. Regarding AF occurrence in *Clarias* sp. and *Oreochromis* sp., the levels were equal to 0.01–2.78 and 0.04–0.4 mg/kg, respectively.

Mycotoxin adulteration of dehydrated marine products is extensive, especially in tropical countries. For instance, in African countries such as Zambia and Nigeria, aflatoxin occurrence in dehydrated marine products has been described in Nigeria as 1.05–25.00 µg/kg [20] and 23 µg/kg [145]. These two countries are known for tropical climates, with high humidity and temperatures throughout the year, and mold contamination recurrently arises.

Table 2 presents an overview of sophisticated detection techniques used to study multiple mycotoxins in seafood.

Table 2. Overview of sophisticated detection techniques to study multiple mycotoxins in seafood.

Country	Sample	Extraction and Clean Up	Detection	Analytes	LOD Range (µg/kg)	LOQ Range (µg/kg)	Validation Parameters	References
Spain	Fish plasma	DLL	LC-MS	AF, OTA, and *Fusarium* mycotoxins	0.1–12	1–17	Linearity, sensitivity, specificity, precision	[57]
Spain	Fish Squid Prawns	LLE and QuEChERS	LC-MS/MS	DON, 3ADON, 15ADON, HT-2, NIV, NEO, ENNA, ENNB, BEA, AFG2, OTA and DAS	0.04–1.5	0.13–5	Linearity, matrix effect, sensitivity, and accuracy	[55]
China	Crucian carp muscles Dehydrated fish products	UAE with solvents and purification with Oasis HLB cartridge	LC-MS/MS	AFB1, AFB2, AFG1, AFG2, OTA, ZEN, T-2 toxin, HT2 toxin and DON	0.1–0.5 0.1–2 0.1–1	0.1–1 0.5–3 0.1–3	Linearity, sensitivity, recovery, and precision	[95]
Norway Spain and France Norway Spain	Atlantic salmon Rainbow trout Sushi Atlantic salmon Gula substitute	Acetonitrile/water and QuEChERS	LC-MS/MS-LIT	AFB1, AFB2, AFG1, AFG2, FB1, FB2, FB3, ENN A, ENN A1, ENN B, ENN B1, BEA, FUS-X, and OTA	1–10	3–33.3	Selectivity, linearity, matrix effect, sensitivity, trueness, and precision	[130]
Spain	Sea bass side streams	UAE with acetonitrile and DLLME	LC-MS/MS-QTRAP	AFB1, AFB2, AFG1, AFG2, ZEA, OTA, BEA, ENA, ENA1, ENB, ENB1	0.05–5	0.2–8	Recoveries, repeatability (intraday precision), reproducibility, linearity, LOD, and LOQ	[141]
Egypt	Smoked herring Canned sardines Frozen fish fillets	Water/acetone	HPLC	AFB1, AFB2, AFG1 and AFG2	-	-	-	[132]
China	Dried shrimp Dried fish Dried mussel	UAE and acetonitrile/water and [n-hexane and immunoaffinity] for clean up	LC-MS/MS	AFB1, T-2, OTA and DON	0.1–0.2 0.1–1 0.3–1	0.3–0.5 0.5–1 1–5	Linearity, sensitivity	[146]
China	Dried fish samples	Acetonitrile/water and UAE	LC-MS/MS	AFB1, T-2, OTA, DON	0.1–1	0.3–3	LOD and LOQ	[2]
Kenya	Smoked, charcoal-grilled, and fresh tilapia muscle samples	Methanol–water and clean up with aluminum oxide	AflatestTM immunoaffinity column method	AFs	-	-	-	[145]
Zambia	Sun-dried kapenta fish	ZearalaTest^{WB}	HPLC/FLD	ZEN, α-ZEL, and β-ZEL	0.003–0.012	0.007–0.015	Linearity, recovery, precision	[38]
Zambia	Dried fish	Ethyl ether–methanol–water	TLC-FSD	AFs	0.005–0.05	0.05–50	-	[147]
Spain	Gilthead sea bream and Atlantic salmon	Acetonitrile: water	LC–ESI–MS/MS	AFB1, AFB2, AFG1, AFG2, OTA, NEO, FB1, FB2, FB3, T-2, DIA, ZEN, NIV, DON, 3-AcDON, 15-AcDON, Fus X, and HT-2	-	-	-	[99]
Spain	Fish (*Dicentrarchus labrax and Sparus aurata*)	Acetonitrile and microwave	LC-MS/MS-LIT	ENA, ENA1, ENB, ENB1, BEA	0.3–3	1–10	Linearity, limits of detection, recoveries, repeatability, reproducibility, and matrix effects	[123]

As Pleadin et al., 2017, found, mycotoxins accumulate in fish organs due to variables such as period of exposure, concentration, fish sex, and species [148]. In addition, due to the high fat solubility of mycotoxin, toxicological examinations on fish found that these compounds are absorbed into the circulatory system of the gastrointestinal tract [96].

6. Seafood Industry and Future Challenges

In its latest report on "The State of World Fisheries and Aquaculture", SOFIA, the FAO underlined that aquaculture and fishery production is at its highest level (214 million tons in 2020) and this field will play a decisive part in the supply of food and nutrition. Concurrently, the global consumption of seafood has increased at an average annual rate of 3.0% since 1961, although pollution, overfishing, poor management measures, and other practices threaten fishery resources [149,150]. In addition, climate change may play a role in the future of the seafood industry. Therefore, GAqPs and SFMs are the key steps in mitigating the effects of mycotoxins in seafood [151]. The contamination of fish and shellfish foods by mycotoxins is a risk to people and animal health and a serious concern in the economic system. Seafood matrices are very complex and, at the same time, very perishable. Avoiding possible health risks from microbial and fungi growth (and possibly developing dangerous chemicals, such as mycotoxins) requires proper handling, processing, preservation, packaging, and storage. Some mycotoxins, such as *Fusarium* toxins, occur in particular during the post-harvesting stages. Among the processing methods, those with higher temperatures may help lower mycotoxin concentration. In general, reduction in moisture level (< 0.70 of a_w) avoids mold development and mycotoxin production. Apart from heat treatment (boiling, canning, and smoking), other methods require reduction in temperature (chilling and freezing), reduction in available water (drying, salting, and smoking), and/or change in storage conditions (modified atmosphere packaging and vacuum packaging) [111,130,152]. For example, some authors identified unhygienic processing and poor storage conditions as the cause of contamination by multiple mycotoxins of dried fish products sold in China. AFB1, T-2, OTA, and DON residuals were detected in 12 of 25 samples [2].

The best way to mitigate mycotoxins in seafood is prevention and control along the production and manufacturing chain. At the same time, monitoring programs and refined analytical methods for these matrices are necessary activities in food safety. These chemical contaminants and other emerging marine pollutants are candidates for future regulations.

7. Conclusions

Mycotoxin contamination in seafood is a cumulative issue that has to be approached by feed producers, crop farmers, researchers, risk assessors and managers, and the authorities. One primary measure that should be employed is to prevent raw materials with high contamination from entering the market or processing them to reduce the potential degree of mycotoxin contamination. Several mycotoxin producers are quiet, so the development of mycotoxins during storage should be investigated more. Consequently, guidelines and recommendations for the proper storage of seafood and operator training should be implemented. Moreover, from this investigation, a scenario of concern exists for fish and shellfish products that may reflect potential health hazards. Further studies on mycotoxins in seafood, new occurrence data, and the evaluation of multi-mycotoxin exposure from different seafood matrices are necessary for proper risk assessment and management and for reducing data gaps.

Author Contributions: Conceptualization, A.M.K.; methodology, S.S., T.D. and S.A.; formal analysis, S.S.; investigation, resources, writing—original draft preparation, S.S., T.D. and S.A; visualization, A.M.K.; supervision, A.M.K.; project administration, A.M.K.; funding acquisition, A.M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

Liquid–liquid extraction (LLE); solid-phase extraction (SPE); solid-phase microextraction (SPME); immunoaffinity chromatography (IAC); liquid chromatography (LC); thin-layer chromatography (TLC); enzyme-linked immunosorbent assay (ELISA); gas chromatography (GC) -electron capture detection (ECD); gas chromatography mass spectrometry (GC-MS); chromatography mass spectrometry (GC-MS); ultra-performance liquid chromatography (UPLC) with photodiode array (PDA) detector (UPLC-PDA); International Agency for Research on Cancer (IARC); European Food Safety Authority (EFSA); tolerable daily intake (TDI); Contaminants in the Food Chain (CONTAM); no observed adverse effect level (NOAEL); low observed adverse effect level (LOAEL); risk management measures (RMMs); rapid alert system for food and feed (RASFF); Food and Agriculture Organization (FAO); Ochratoxin (OTA); Enniathin A1 (ENNA1); Enniathin B (ENNB); Enniathin B1 (ENNB1); T-2 toxin (T-2); Aflatoxins (AFs); Aflatoxin B1 (AFB1); Deoxynivalenol (DON); limit of detection (LOD); liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS); not available (NA); high-performance liquid chromatography fluorescence detector (HPLC-FLD); chloroform ($CHCl_3$); ethyl acetate (EtOAc); quick, easy, cheap, effective, rugged and safe (QuEChERS); dispersive solid-phase extraction (d-SPE); ultrasound-assisted extraction (UAE); liquid chromatography system coupled to tandem mass spectrometry with a linear ion trap (LC-MS/MS-LIT); high-performance liquid chromatography (HPLC); relative standard deviations (RSDs); thin-layer chromatography with fluorescence scanning densitometry (TLC-FSD); good aquaculture practices (GAqPs); sustainable fishing methods (SFMs); a_w: water activity.

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