Integrated mapping of existing solutions to prevent insect infestation, mycotoxin contamination, pathogen development

[31-10-2022]

TRACE-RICE with Grant nº 1934, is part of the PRIMA Programme supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation

PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

http://trace-rice.eu

Project Acronym: TRACE-RICE

Project Coordinator: Carla Moita Brites

Project Duration: September 2020 – August 2024 (48 months)

Deliverable No.: D2.1 Integrated mapping of existing solutions to prevent (i) insect infestation, (ii) mycotoxin contamination, (iii) pathogen development. (M6, INIAV)

Dissemination level: Confidential

Work Package: WP2 - TEST SOLUTIONS AND BEST PRACTICES TO REDUCE CHEMICALS

Task: 2.1 SOLUTION MAPPING

Lead beneficiary: INIAV

Contributing beneficiaries: CSIC-IATA, EM, iBET

Due date of deliverable: 28 February 2021

Actual submission date: 31 October 2022

HISTORY OF CHANGES			
Date	Beneficiary	Version	Change
02-02-2021	CSIC-IATA	1	Published paper related with bacterial contamination and predictive solutions
19-09-2022	INIAV	2	Published paper related with Mycotoxins Contamination in Rice
18-09-2022	EM	3	Report of WP2
31-10-2022	INIAV	4	Final Version approved by project coordinator

1. Purpose

The main goal of Task 2.1 is to mapping the solutions to addresses 2 major problems that occur in the storage and handling of rice from farm to fork and that have serious impact on the value of the product and significant potential for public health hazards, resulting from the consequence of insect infestation and microbial contamination. The solutions must be evaluated in operational and financial terms in order to ensure that the selected one for testing will focus on the most promising solutions in real-life scenarios. It is also important to note implications of some of these solutions with a life-cycle view (for instance, in the use of circular economy approaches for protective packaging solutions).

Considering the 3 sub-tasks of DoA (2.1.1, 2.1.2, 2.1.3), the following attached review articles were published:

SUBTASK 2.1.1 INSECT AND FUNGAL GROWTH MITIGATION: *Advances in environmentally friendly techniques and circular economy approaches for insect infestation management in stored rice grains.* In this review it was possible to verify that there are several examples of alternative and non-chemical treatments that are promising to investigate their effectiveness for applying at an industrial scale.

SUBTASK 2.1.2 MYCOTOXINS AND PESTICIDES RESIDUES-*Mycotoxins Contamination in Rice: Analytical Methods, Occurrence and Detoxification Strategies.* Compilation of the most relevant studies and review the main methods used in the detection, quantification and detoxification strategies of mycotoxins in rice.

SUBTASK 2.1.3 BACTERIAL CONTAMINATION AND PREDICTIVE SOLUTIONS-*Risk of Bacillus cereus in Relation to Rice and Derivatives.* Review from the perspective of risk assessment of the risk posed by B. cereus to the health of consumers and of some control measures that can be used to mitigate such a risk.

foods

MDPI

Review

Advances in environmentally friendly techniques and circular economy approaches for insect infestation management in stored rice grains

Inês Gonçalves de Sousa [1,2], Jorge Oliveira[3], António Mexia[2,4], Graça Barros[2,4], Carina Almeida[1], Carla Brazinha, Anna Vega[5], Carla Brites[1,6*]

[1] National Institute for Agricultural and Veterinary Research (INIAV), I.P., Av. da República, 2780-157 Oeiras, Portugal;
[2] LEAF - Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal;
[3] Ernesto Morgado S. A.; Rua Prof. Casimiro de Oliveira 21, 3090-833 Barra, Portugal;
[4] Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal;
[5] Grain Technik Pvt. Ltd., B-2/12, Mohan Co-operative Industrial Estate Badarpur, 110044 New Delhi, India; anna.vega@graintechnik.com
[6] GREEN-IT Bioresources for Sustainability, ITQB NOVA, Av. da República, 2780-257 Oeiras, Portugal; carla.brites@iniav.pt

* Correspondence: carla.brites@iniav.pt

Abstract: Rice (*Oryza sativa* L.) is a staple food for about half of the world's population. Therefore, it is extremely important to investigate solutions that minimize losses and production costs for producers and ensure food quality and safety for consumers. Chemical methods are used to prevent rice losses due to infestations, however, it is urgent to change this situation, as it is harmful to the environment and human health. Some alternative methods can be current petroleum-based packaging with modified atmospheres, radiofrequency, biopesticides, essential oils from plants, among others that it is necessary to study their effectiveness and economic viability. Considering the bio-packaging produced from rice by-products (rice bran, rice husk/hull and rice straw) reported in literature, and according to the principles of the Circular Economy, it is recommended an integration of the best selected treatments/solutions for insect management with the bio-packaging from rice by-products. In this review it was possible to verify that there are several examples of alternative and non-chemical treatments that are promising, so it is important to investigate more for obtain effective technological solutions that can be applied at an industrial scale.

Keywords: rice, non-chemical, treatments, prevention

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Foods* **2022**, *11*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Firstname Lastname

Received: date
Accepted: date
Published: date

1. Introduction

Rice wastage occur at every stage of the production process, from the farm to the consumer's homes (Qu et al., 2021). Also, there is an increasing concern to continuously improve food production and minimize crop losses, as it is necessary to respond to the demands of the growing population. In this way, it is very important to understand the rice wastage origins and minimize rice losses that occur due to insect infestation.

Occurrence of damages and losses in rice stored can be direct (physical loss of grains) or indirect (loss in quality and nutrition) (Mesterházy et al., 2020). Beyond that, insect pests are known by promote the weight loss, which is very important factor because it causes nutritional value loss, commercial loss, and quality degradation (Zulaikha & Yaakop, 2021). Insect damage can be significant when rice is stored for long periods of time and insect populations reach very high levels. The increase in temperature and water

content results from the activity of insects and can promote the deterioration of the grains. However, these damages are difficult to estimate and are affected by several factors, such as temperature, relative humidity, atmosphere conditions and storage duration, as well as the intrinsic properties of the various rice varieties. Therefore, due to inappropriate storage conditions, rice can be an ideal substrate for several contaminations of larvae and/or live insects.

Post-harvest procedures have as main objective to maintain the quality of the grain, preventing the contamination by insects or fungi. Therefore, the choice of that procedures can strongly influence rice quality (Müller et al., 2022). If rice is maintained stored in cool temperatures and dry conditions, eggs that might still exist would not hatch, hence the advice to store rice in cool and dry conditions. However, a rice miller has little control over storage conditions in client's warehouses and consumers homes.

Meanwhile, the use of pesticides in field and grain storage units to control infestations is still common, mainly chemical agents (commonly fumigations) during storage. Usually, organophosphates and pyrethroids are the most used insecticides, due to their effectiveness against insects (Yao et al., 2020). The pesticides are being used worldwide but their overuse is causing serious environmental problems, such as the development of insect pests' resistance (Lee et al., 2003). Also, the chemical residues affect public health (Chou et al., 2022). The fumigations generate residues of chemical contaminants that compromise the natural quality of rice, and its products and consumers are increasingly concerned about insecticide residues in food products (Lee et al., 2003).

In line with the advancement of insect resistance mechanisms, there is a growing increase in restrict the use of chemical pesticides, being replaced by alternative solutions in the management of insect pests in stored food products, to protect the food quality and the environment. Biopesticides appear as novel eco-friendly tools and an adequate implementation of them could generate a great alternative to protection of stored grain against pests' agents (Herrera et al., 2018).

The search for solutions to prevent insect infestations and the evaluation of their effectiveness are therefore priority aspects for the sustainability of the rice value chain and which may also have an impact to a large extent on food products derived from rice and other cereals.

The objective of this review was to analyse all potential alternative solutions that have been proposed in scientific and technical literature, to then select the most promising approaches to mitigate losses due to insect infestation.

2. Importance of cleaning the grains and the equipment

Cleaning the rice grains and the equipment is a common postharvest treatment. Rice cleaning is extremely important during the harvesting process, which function is to separate the stems and weeds from the rice grains mixture (Zhao et al., 2022).

When prescribed as a quarantine treatment, it is usually followed by inspection by the importing regulatory agencies to ensure that the cleaning was successful in removing undesired pests. However, cleaning alone may be insufficient and often needs to be followed by another treatment.

3. Rice insect infestation

The origin of pests as well as the intensity of their attacks and the damage they can cause are considered important aspects for the defence of stored products.

Insect infestation is a major cause of produce loss. However, the presence of insects in rice is nearly inevitable and the deterioration of rice can start before harvest, from insects that attack in the field. Inside the grains, insect movement can be determined by

seasonal conditions and grain temperature. This means that, in the months when temperatures are higher, insect infestations will occur more on the surface of the grain, while when temperatures are low, infestations will occur more in the centre of the grain. In the latter situation, insect infestations may not be detected early, until the insects are present in large numbers (Hagstrum et al., 2012).

All stages of the insect life cycle occur inside the grain. Some species of common insects identified in rice grains are rice weevil (*Sitophilus oryzae* L.), granary weevil (*Sitophilys granaries* L.), lesser grain borer (*Rhysopertha dominica* F.), and angoumois grain moth (*Sitotroga cerealella* O.), and they are considered primary pests (Hagstrum et al., 2012). These insects that occur during rice storage can develop inside the grain (internal or hidden infestation), consuming the endosperm, or develop outside the rice grain (external infestation), feeding on the bran, dust, or broken grains.

The rice weevil and the rice moth are the most relevant indoor infestations for stored rice and are part of a set of insect species that pierce the grains and reduce them to flour. *Sitophilus oryzae* and *Sitophilus granarius* are the most common and are quite similar, but each has unique physical characteristics and capabilities. Adult weevils have long snouts with chewing mouth parts at the end. A female that has been fecundated will chew a hole in the kernel with her long snout, excavates a small cavity into which she places an egg and then seals the hole with a gelatinous plug. An infected kernel is almost indistinguishable with the naked eye. Normally, only one egg will become larvae per kernel, but an adult female can lay about 400 eggs in her lifetime. The rice weevil usually lays more eggs than does a granary weevil. Eggs hatch in few days under favourable conditions but may lay dormant for a long while until such conditions are set (namely temperature and humidity). The complete metamorphosis from egg to larvae, then pupa and then adult can occur inside the kernel in 35 to 40 days and the adult then chews its way out. The rice weevil is one of the most widespread and destructive insect pests found in stored cereals throughout the world, and the interaction with rice involves all life stages of the insect. These insects cause losses in cereals and affects the quantity and quality of the grains (Mesterházy et al., 2020). This specie can also infest the rice in the field and usually turn that commodity into flour.

Rhyzopertha dominica is also one of the most damaging insects that normally appear in rice grains. Infestations caused by these insects are difficult to detect since larvae and pupae develop inside infested grains (Mancini et al., 2007). The chances of infestations by this insect on stored rice are increased as this insect has the ability to fly easily. Both adults and larvae of *Rhyzopertha dominica* feed on rice grains and, in the case of large infestations, the grain can develop a musty odour and may also become heavily soiled with excrement.

Sitotroga cerealella is the most abundant grain moth in the storage of paddy rice. Usually, moth infestations affect in the upper layers of stored grains in bulk, which limits the direct losses that this insect can cause. These insects can also infect the grains in the field and most of the damages are provoked by larvae inside rice grains (Mancini et al., 2007).

4. Detecting and Monitoring tools

The search for solutions to detect and monitor the internal (hidden) infestation is of greater relevance for the industry because the grain is normally stored with husk (paddy rice), whose structure provides protection against external insects.

Regarding the techniques to inspect grain for internal infestation, the most conventional ones resort to the methods described in ISO 6339-4:2015 (REF) describing a total of 5 methodologies for estimating the degree of, or detecting the presence of, hidden insect infestation. These include: the determination of carbon dioxide production; the ninhydrin method; whole grain flotation method; acoustic method and X-ray method. The principles of these methods will be explained bellow, and we will also provide information in recent advances that can improve the performace of the different tecniques.

Starting with the determination of carbon dioxide, this method is based on the fact carbon dioxide figure is largely a measure of insect infestation (as a result of the insect respiration rate) *(Howe & Oxley, n.d.)*, considering that the metabolic rate of dry grain is very low. It has been shown that mature larval instars of grain weevils produce more carbon dioxide than adults and that accumulation of carbon dioxide of infested grain sample during a period of 24 hours is easily measurable by atmospheric sensors (Xmgwei et al., n.d.). Nontheless, it is also known that moisture content and temperature can interfer with carbon dioxide release in grain, specially because they can potentiate the grow of fungi, such as *Aspergillus* spp, which can produce significant amounts of carbon dioxide (suprior to the amounts produced by insects) (Fleurat-Lessard, 2011; Zhai et al., 2015). As such, increases in carbon dioxide can often be a result of fungal spoilge instead of hidden infestation. Advances on carbone dioxide determination have been made reagarging wireless, in locus, sensors that resort to machine learning algorithms for a real-time monitoring and early warnings on possible grain infestation/spoilage *(Singh & Fielke, 2017)*.

As such, recent sensor can help predicting detecting with good accuracy incipient or ongoing spoilage/infestation.

In what concern to accustic methods, these rely on the identification of sound patterns of targeted insects. An acoustic vibration sensor, connected to an amplification system, will transmits the noise from the feeding activity of hidden insects. The use of acoustic technology in insect pest management applications has increased significantly between 1980 and 2010 (Mankin et al., 2021). Currently, different acoustic devices are commercialized for detection of hidden insect infestations (Banga et al., 2018; Mankin et al., 2021). Advances on this systems include digital signal processing and statistical analyses tools, such us those based in neural networks or machine learning/deep learning, to distinguish targeted pests from each other and from background noise (Santiago et al., 2017; Rabano et al., 2018). These advances enable an accurate automated monitoring of the abundance and distribution of pest insects in stored grains, which might have a great impact on the value of future commercial solutions.

Ninhydrin method is based on a colorimetric reaction of ninhydrin, originally yellow, with a free α-amino group of primary amino acids, producing a purple colored dye known as Ruhemann's purple. As such, when an infested dry grain is crushed, the amino acids from the insects body fluid will react with ninhydrin in thea paper surface, originatying a purple spot. Amino acids of the grain are not released and do not react. The number of spots indicates the level of hidden infestation (REF ISO). Not much developments have been reported on this method, and this tecnquine is less ameable to automated, in loco application; nontheless new method adaptation have been proposed to increase accuracy regarding the detection of worm's ovum and low instar larvae (https://patents.google.com/patent/CN105806843A/en).

Whole grain flotation method relies on the fact that internal insect infestation reduces the mass of grain, making the grains to float. When sound and infested grains are immersed, the sound ones will just sink, while the infested will float to the surface. The floation method has found a good level of implementation wordwide and adaptations of this method have been reported that allow the detection of insect fragments in bran, fine bran and flower (Fu et al., 2021; Germinara et al., 2010). Nonetheless, this method is time-consuming and results are of qualitative value, but not of quantitative value, mostly because the method is most likely to produce an underestimate of the level of infestation present in the sample.

Regardign X-ray imaging tecniques, these are based on the exposute to soft X-ray of a one-grain thinkness layer of rice, followed by inspection to identy insects within the grains. There are many advantages on using X-ray since it is a fast, non-destructive and

accurate technique for internal and external detection of insects, regardless of the life stage of the insect. Furthermore, recent algorithms focused on X-ray image contrast enhancement, or microcomputed tomography for 3D imaging, enable superior diagnostic images and, consequently, high accuracy *(Shah & Khan, 2014; Srivastava et al., 2020)*. Nevertheless, the automatic inspection of insect infestation is still a challenge. On this regards, deep learning, in particular artificial neural networks and convolutional neural networks, has been applied to discriminate infected and non-infested maize grains; and this knowledge is likely to be translated to rice infestation (Boniecki et al., 2020; da Silva et al., 2021).

In addition the most conventional tecniques, other efforts have been put into tecniques that have the potential to optimize the test-time, improve accuracy or/and to apply non-destructive tecquines. NIRS (near infrared spectroscopy) analysis or the, profile/presence of specific volatile compounds produced by specific insects (wich analysis can be performed by chromatography or mass spectrometry techniques, after solic phase extraction; or, more recently, electronic noses that sense specific volatile compunds) are good examples of recent strategies/techniques that have been applied to this research field that that can be review by Banga et al., 2018.

Molecular- based tecquines, resorting to the detection of specific genetic regions of a target species, are another good exmaple of recent developments for their ability to identify the causing agents. Those tecniques have emerged in the last decades due to their accuracy, limit of detection, specificy and high throughput capabilities. Among the diverse tecnologies, polimerase chain reaction (PCR)A detection tecquines, in particular those based on quantitaive approches (qPCR), have gain great relevance. However, although PCR is routinely used in food industry to detect foodborne pathogens or genetically modified organisms (GMOs) in foods, its use to detect insects presence is still at an early stage. Regarding grains/cereals infestation, there are a few reaserch works that resort to PCR and insects species-spcific DNA regions to accuratly detect, and even quantify, early infestation (Negi et al., 2021). Examples can be found for both internal/hidden and external infestation and for different grains/samples.

A study conducted by Nowaczyk et al. (2009) on the development of a real time PCR method for detecting Tribolium confusum infestations in stored products, has shown the method ability to detect as low as 1 insects per kg of oat flakes. Also, the detection of *Tribolium castenaum* external infestation by a quantitative (qPCR) method has also been validated in wheat flour, showing a detection limit of 0.046 adult insects in 5 g of wheat flour (Negi et al., 2021). These works clearly show the potencial of this tecnique to quantify infectation to very low levels. Considering that FDA has defined a maximum permissible limit of insect fragments in flour of 75 insect fragments, or approx. 3 adults, per 50 g of flour (Negi et al., 2021), the value of PCR tecquines becames quite clear.

Back in 2016, a real time PCR method has been developed to identify hidden infestations of *Rhyzopertha dominica* in grain (rice, maize and wheat) (Solà, 2016). Later on, that study has evolved to include the five most relevant internal pest. Thus, in the work of Solà et al. (2018), we can find the most paradigmatic example of the PCR potential for detecting hidden infestation. A multiplex PCR was deleloped and tested in different grains, including rice. Insects species included *R. dominica*, *Sitophilus granarius*, *S. oryzae*, *S. zeamais* and *Sitotroga cerealella*. The estimated detection limit was found to be 0.1 pupa/kilo of rice, except for *R. dominica* (10 pupae/kilo).

As such, taking into consideration the specificity and limit of detection of this detection tecniques, as well as the broad distribution/use of PCR instruments in food-related laboratories, it is expected that industry can resort more often to this techniques. Nonetheless, their potential for application in loco for a real-time monitoring is limited, as they require laboratory settings. Because of this, methodologies such as those based on acoustic or carbon dioxide sensors, which are more amiable to in loco automated monitoring, are expected to find a higher degree of dissemination in industrial settings.

5. Control measures

5.1. Fumigation methods: limitations and health concerns

Fumigation is a chemical treatment and one of the most effective methods for the control of insects' growth in stored cereal grains (Ref[a]). This method can be used against several species of insets and is unexpensive (Paul et al., 2020). If properly applied, the treated rice would remain in a hermetic container (or silo) for sufficient time to kill living insects as they are formed but fumigation will not destroy the eggs. As most likely infection has already occurred on the field, rice producers and millers could only rely on receiving the rice, fumigate it properly, hope that all live insects die and all eggs turn to larvae, pupa, adult and then die. Rice kernels damaged by the infection will be removed in the sorting process.

Despite the success of fumigation, resistant insects have been identified (Donahaye et al., 2007) and this implies that over time the insect species becomes more tolerant of the chemicals that thus lose efficiency (Ref[a]). Furthermore, fumigation is not always properly applied, for instance, an Australian Grains Research and Development Corporation (GRDC) survey carried out during 2017 revealed that only 49 per cent of growers using phosphine applied it correctly — in a gas-tight, sealed silo.

The fumigants applied to control pest populations, can have negative effects and be toxic to humans (Paul et al., 2020), and some stored-product pests have developed resistance to them (Donahaye et al., 2007). Organophosphorus and pyrethroids are widely used to control various pests because they are unexpensive and have high efficiency, however, organophosphorus have provoked pollution to the environment and ecosystems, leaving toxic residues in water and soil, which can easily enter in the food chain. Pyrethroids contain synthetic chemicals derived from modification of pyrethrin obtained from flowers, there being several pyrethroids used in agriculture.

A deltamethrin incorporated polypropylene bag (ZeroFly® Storage Bag) has been developed (Vestergaard SA, Lausanne, Switzerland) for which it claimed great potential to reduce postharvest losses of cereal grains and grain legumes (P Anankware et al., 2014). This compound However, the study found that the bags were indeed efficient to present insects from entering the bags, but seemed to leave those already in unaffected, so its usefulness is doubtful. k-obiol is other pesticide produced by Bayer®, which is being used in industry recently as an alternative to phosphine. Unpublished trials though were unconvincing regarding its performance for fumigation compared to phosphine. Its impregnation in packages would therefore likely give dubious results as well.

However, the continued use of these pesticides increases the risk of exposure (Yao et al., 2020). In fact, there has been an increasing concern worldwide in the substitution of chemical treatments for methods of biological origin, since chemical pesticides have tremendous impact on biodiversity, environmental, animal, and human health. To address this necessity, it is important to identify and experiment realistic solutions that could minimize the use of insecticides and reduce their impact in the environment (Ali et al., 2017).

5.2. Control by environmental parameters

Critical environmental parameters such as temperature and atmosphere (extrinsic factors) affect the storability of rice because can cause problems related with insect infestation and other biological contaminations. Therefore, temperature and CO_2 control is highly important to prevent rice losses due to those problems and to estimate the greatest risk periods (Mancini et al., 2007).

5.2.1. Temperature

Grain temperature is one of the most important factors to control insect infestation and monitoring the temperature of rice grains is mandatory for maintaining quality throughout storage (Li et al., 2018).

Temperature control is an effective method to prevent insect infestation in rice storage (Champagne, 2004), since insects can't survive or thrive outside a temperature range of 13 – 35 °C (Fields, 1992). Although treatments using high temperatures are possible for controlling pests in stored products, they can lead to quality degradation (Paul et al., 2020). Bringing the grain temperature to a level, where heat treatment is effective using hot air is very difficult, since the grain would dry, and the evaporative cooling would keep the rice temperature much lower than the applied air temperature. Heat treatment with hot air hence would lead to an undesirable, substantial overdrying of the grain. Furthermore, any kind of isochoric heating of the grain, that would have a positive effect on insect infestation issues, would significantly change the product properties and lead to discoloration triggered by the so-called Maillard Reaction (Bhattacharya, 2011), as is observed, and in these cases desirable, during paddy steaming and parboiling.

On the other hand, cooling rice, with or without refrigeration, has been shown to be effective against insects (Champagne, 2004). There are many storage situations where ambient conditions are not sufficient to cool the grain, hence refrigerated air units for chilling grain have been developed in answer to these situations (Maier & Navarro, 2002). In fact, the chilled aeration of grain has been successfully utilized to preserve grain quality and has already been applied commercially during the past 60 years (Maier, 1994). Maintaining low temperature- and moisture levels in bulk-stored grain was identified in a major study on "Enhancing the quality of U.S. grain for international trade" (U.S. Congress, 1989), as the main way to preserve grain quality, and to prevent damage from moulds and insects as early as 1989. Today, grain chilling is the most used technology in the rice industry to remove the excess of heat after harvest or drying, significantly preserving the quality of the rice stored and allowing long-term storage regardless of the ambient conditions.

In grain chilling, grain is cooled using a mobile refrigeration system that controls both the temperature and relative humidity of the aeration air independent of the ambient conditions (Maier, 1994).

Although storage temperatures of as low as 5°C have been recommended in literature (Katta et al., 2019), it has been shown, that keeping the grain temperature below 20°C already significantly reduces the development rate of insects when compared to 25°C product temperature (Morales Quiros, 2017).

Several studies could proof, that the use of grain chilling in industrial silo complexes can keep the stored paddy insect free, even for extended storage periods and extreme weather conditions, if the product temperature is kept at 15°C or below. Lazzari et al. (1994) found that chilling of a 5,000t metallic paddy silo to 15°C in Brazil controlled the insect populations for about 60 days without the need of re-chilling. Similarly, Lazzari et al. (2010) reported, that after initial chilling to 12-14°C, stored rice in a huge rice facility in Brazil, kept its temperature about 60 days without re-chilling. Keeping the rice at this temperature level, it was found free of external insects after 8 months of storage.

These studies furthermore highlight the fact, that once the grain has been initially cooled, "only occasional re-chilling for short time periods is required to maintain chilled storage conditions due to the insulating properties of the grain itself" (Maier, 1994).

A simulation carried out for a paddy silo in Costa Rica (Morales Quiros, 2017) revealed, that it would take less than 5 days to cool the product to a top layer temperature of 14.6°C and that once cooled, the average grain temperature would only increase to 15.5°C, over a storage period of 6 month despite the high local ambient temperatures. Since the average grain temperature remained within the range in which insect development would stop (Fields, 1992), the need of chemical control of stored-product insects would reduce or be eliminate completely.

Chilling grain below 15°C in less than a week avoids that most insect species complete even one life cycle because most of them take at least a month to develop from egg to adult at ideal temperatures between 30°C and 35°C (Rees, 2004). Morales Quiros (2017) concludes, that "chilled aeration is the only technically feasible strategy to achieve average grain temperatures sufficiently low to reduce or eliminate the need for chemicals to control stored product insects".

Due to the fact, that after initial chilling only occasional re-chilling is required, grain chilling could be proven to be an economic solution for chemical free pest control even in tropical conditions. Morales Quiros (2017) found the operational cost of grain chilling to be lower than for the combined cost of aeration with ambient air and fumigation combined. Even in moderate climates in Europe, the use of grain chillers can be cheaper than the use of aeration fans, when the weather is unfavourable for longer time periods (for example during the unusually wet summer 2021 in southern Germany), given the extremely long time to bring down the grain temperature using aeration fans in this case.

It is furthermore important to note, that chilling not only hinders the growth of pest populations, but also allows to avoid quality losses in cereal storage and the deterioration of the product (Mancini et al., 2007).

Lazzari et al. (2010) however highlighted the importance of proper cleaning of the storage facility before storage for a successful chemical free and insect free paddy storage using a grain chiller. A one-time phosphine fumigation cycle followed by grain chilling to 15°C, is widely used in the industry, if living insects are present before storage and has proved to be effective for insect free long-term storage. It is however important to remember, that phosphine fumigation is not effective at low temperatures and hence has to be carried out, before the grain is cooled.

It can be concluded that grain chilling is an economical and efficient method for insect control even during long term storage and independent of the ambient conditions, that furthermore preserves the quality and quantity of product stored.

5.2.2. Atmosphere

The control of the atmospheric composition for the protection of grain stored products, such as rice, has been extensively reported (Carvalho et al., 2019; Covele et al., 2020; Guenha et al., 2014; Martin et al., 2015). In fact, insect eggs would not hatch under certain conditions such as the absence of oxygen. Therefore, whether in a silo or in a small consumer package, if the environment is hermetic and has no oxygen, infestation will not occur.

There are a few options for control the rice grain surrounding atmosphere.

- **Vacuum packaging.** All air is removed, the packaging material ensuring hermeticity will also protect from humid storage environments (high water vapour barrier) and therefore infestation will not occur all the way to the final consumer. This is a bit more expensive than normal packaging because the packaging material must be more resistant and requiring a vacuum packaging machine, but this is an efficient option to prevent the growth of insects.
- **Hermetic packaging.** This option requires a more expensive packaging material than usual, with a very high barrier to gas permeance, being cheaper than the option above as it would not require the vacuum packing. This technology avoids the interactions with the surrounding environment and in addition to maintaining food quality, it can extend shelf life during storage (Carvalho et al., 2019). If eggs would be present, their development would consume the oxygen and then the growth would stop. This is known as passive Modified Atmosphere Packaging (the modified atmosphere is created by the metabolisms ongoing in the product itself). This has been tested for instance by Guenha et al. (2014), concluding that the use of hermetic packaging is safe, pesticide-free, and sustainable. It also contributed to a decrease in

insect infestation. A particular type of bag (PICS - Purdue Improved Crop Storage bags), consisting of two inner layers of high-density polyethylene and an outer layer of woven polypropylene, was reported to give excellent results by Martin et al., (2015). In this case, the results proved that wheat grains stored in the PICS bags had lower levels of insect damage, comparing to conventional packaging. Covele et al. (2020) also studies hermetic containers as an alternative to preserve rice grains, since this method proved to be efficient during 12 months without being necessary the use of pesticides. The results showed that this could be a green alternative for safe storage of rice with several advantages.

- **Active MAP.** Active Modified Atmosphere Packaging consists of removing the normal air and injecting a different gas composition instead. It also obviously requires a hermetic package, so the cost of this solution is higher than even vacuum packaging, as one has to add the cost of the gases. However, there are some cost-effective solutions to generate a modified atmosphere to inject into the packages. Several options have been reported in this regard:
    - **Carbon dioxide.** Carvalho et al. (2012) reported a comprehensive trial of use of CO_2 enriched atmospheres (90-95%) in actual storage silos and big bags. The modified atmosphere very successfully eliminated infestation. CO_2 would also have anti-fungicidal properties, thus providing an additional benefit for that equally important cause of losses during storage at rice milling companies. Their treatment suppressed insects in the state of egg, early larvae, and adult. Thus, can be applied modified atmosphere technologies either in the final product, during the packaging process, or in other stages of the process to preserve the quality and flavour of rice during storage, as they are safe and environmentally friendly tools (Carvalho et al., 2012).
    - **Ozone.** Ozone gas can be used for disinfestation and decontamination, since it does not produce residues (Paul et al., 2020) and has important advantages comparing to conventional food preservation methods (Savi et al., 2020). Ozone would be used as fumigation in silos, it is not suitable for packaging because ozone decomposes quickly and therefore it is necessary to keep generating to maintain its concentration. Its use is described in Amoah and Mahroof (2019), for instance. The results reported by these authors are not very encouraging. While ozone can affect all stages of the insect life cycle, it very much depends on how deep in the kernel the egg was located, as the ozone effect is rather limited to the surface and close to it. Even with treatment for 60 hours with high ozone concentration, at depths of 15 cm and higher there was still significant survival. Rice kernels are much smaller than this though, so the treatment could be quite effective if applied in a fluidized bed for such time as needed for all eggs to eventually be destroyed as they hatch. Ozone also has some disadvantages as a stored product fumigant as it is a strong oxidizer and the effect of ozone exposure on silo materials needs to be assessed. It may increase corrosion rates on metal components and degrade equipment such as rubber seals, and electrical equipment at unacceptable rates. There are also some reports that the ozone gives an off taste to the rice (Ref[a]). Not directly related to contamination by insects but with the residues of chemical treatments, de Ávila et al. (2017) studied ozone gas as degradation agent of pesticide residues in stored rice grains. The samples of rice treated with insecticides were exposed to the gas, being that after ozonation the quality

of rice grains were not affected, and the technique was promising to remove insecticide residues in rice grains.

- **Nitrogen.** Total removal of oxygen while maintaining nitrogen instead of vacuum has been proposed (Ref[a]) to implement in silos by using pressure-swing adsorption to gradually replace normal air by an environment rich in nitrogen (above 98%), extracting oxygen from the kernels themselves. It would have no advantage over using CO_2 except that prices are a bit lower, but it would not have the same anti-fungicidal effect. However, it is perfectly inert and give no change to the organoleptic quality of the rice (Ref[a]).
- **Silica.** Silica has given good results in preventing insect development in cereals (Debnath et al., 2010; Kar et al., 2021). It has been proposed initially by using cheap inert dust, like volcanic ash which is high in silica (over 50%). However, there would be issues with the residues left from the dust that would now become part of the rice, which includes significant potential for off-flavours and insoluble particles to float as rice is cooked. Thus, Kar et al. (2021) proposed a nanotechnology approach using silica nanoparticles. The treatment was considered effective, but there was residual presence for all treatment conditions reported.

5.3. Natural oils and biopesticides in packages

Natural oils and biopesticides are some examples of chemical substitutes that can be used to control and prevent rice losses due to insect infestations.

In fact, natural oils could be an excellent alternative treatment to prevent rice biological contaminations. Some natural oils extracted from several plants have been shown to possess significant antifungal and repellent properties, and insecticidal activity against stored-product pests. Natural products that would be organoleptic acceptable could be mixed with the rice and offer an insecticide type of protection. A few options have been reported in this regard:

- **Spice oils.** Garlic has well known properties against insect infestation. However, its use as is would concede strong flavours to the rice. Essential oils obtained from garlic, ginger, black pepper, and fennel could be used instead, providing less organoleptic impact. Their effectiveness was reported by Chang et al., 2017, placing these different types of oil extracts in sachets instead of mixing them with the rice, to avoid flavour impacts. Sensory assessment proved no organoleptic impact. However, the fumigation capacity was just around 80% at best.
- **Basil oil.** Basil oil has proven effective in killing rice weevils in open air and thus suggested as a potential means to control infestation. However, Follett et al., 2013 reported low effects on weevil mortality and reproduction rate when applied in packed rice.
- **Cinnamon oil emulsion.** Shi et al. (2022) have just claimed very high efficiencies in preventing rice weevil infestation by using an emulsion of cinnamon oil, which prevents its otherwise rapid oxidation and loss of toxicity. The emulsion used anhydrous ethanol and there was no analysis of potential organoleptic impact though.

In addition to those already mentioned, there are other studies about natural oils extracted from plants that had activity against insect's metabolism. Guettal et al. (2020) concluded that the natural oil from *Citrus limonum* exhibited fumigant toxicity against *S. granarius* adults, confirming its potential as a natural alternative to synthetic insecticides for the control of stored-product pests. To study the fumigant toxicity of *C. limonum* essential

oil, after washing, the leaves were dried in the shade and ground into powder. Then, the obtained oil was dried over anhydrous sodium sulphate and was analysed by gas chromatography mass spectrometry (GC-MS). The components were identified based on retention index that was compared with the reference mass spectra.

The orange oil has also been used as an alternative agent for controlling many insect pests since it is neurotoxicity to insects while in a study developed by Chou et al. (2022) it showed low mammalian toxicity and short environmental persistence. In addition, Mishra et al. (2013) concluded that essential oils of *Syzygium aromaticum* and *Aegle marmelos* could be recommended as an alternative to synthetic insecticides, since they are inexpensive, easily available at farm level, environmentally sound with low mammalian toxicity. Finally, Bhavya et al., 2018, shows that essential oil of *O. tenuiflorum* had a significant fumigant activity against *S. oryzae,* concluding that this essential oil could be used in the formulation of biofumigants as a safer alternative to chemical fumigants.

Al-Harbi et al. (2021) evaluated the insecticidal activity of *Ocimum basilicum*, *Nigella sativa*, and *Lavandula angustifolia* essential oils against *S. oryzae*, by assessing mortality percentage assay in the adult stage of the insect, as well as analysing genes associated to toxicity effect the natural oils.

Biopesticides are frequently part of the natural defence mechanism of many plant species, usually showing high selectivity against target pests with low toxicity, besides being biodegradable. They can be applied to protect crops and seeds, which can be seriously damaged by insect infestation during storage and transport, causing important economic losses (Goñi et al., 2017). The use of biopesticides is increasing since regulation agencies are setting lower residue limits for synthetic pesticides and encouraging the use of no synthetic alternatives (Codex Alimentarius, 2018). Also, consumers are encouraged for the replacement of chemical substances by biopesticides.

Biopesticides can be impregnated in the packaging material in order to create an anti-insect effect and avoid significant changes in the rice grain composition. This may work in killing adult insects, but it must be noted that the dead insects will not disappear from the inside of the package. The insecticide is released from the package over a given period of time. This is the critical issue for application to rice weevils, as an egg can take over a month to become an adult, which is then killable. Some options have been suggested in literature:

- **Terpenes.** Goñi et al. (2017) impregnated low density polyethylene films with supercritical CO_2 and obtained a good result in 100% mortality of adult insects but only for up to two days, with the toxicity decreasing to very low in just seven days. These researchers developed these films as a strategy for developing a packaging material for protecting seeds, kernels and derivatives during storage and transport. Although this study was related to maize and its pests, it had a positive effect, and it would be important to verify its effectiveness on stored rice.
- **Biopesticides in double layered bags.** Soujanya et al. (2018) proposed to place a biopesticide in between 2 layers of plastic for a double layered bag with the biopesticide not being in contact with the rice. The biopesticide used in this study was leaf powder of *Tinospora cordifolia*. Reported results show a good efficacy, despite not being 100%.
- **Chitosan.** Silva et al. (2022) reported some fungicidal effect of chitosan coated paperboard. The best efficiency in preventing insect infestation was however under 80%.

The development of active packaging with incorporation of biopesticides is an innovative technology for food preservation considering their antifungal, insecticidal, repellent, and herbicidal activities. Herrera et al. (2018) obtained a bioactive material for stored grains protection by incorporating 1-octen-3-ol in low density polyethylene films. The supercritical CO_2-assisted impregnation of LDPE films with that biopesticide was carried

out in a high-pressure cell, with magnetic stirring and using a high-pressure impregnation system. The films developed by these authors indicated that this biopesticide has insecticidal activity against *S. zeamais*, since the results showed 100% of mortality after 24 hours. Although *S. zeamais* is a maize pest, it is also common in stored rice, and it would be important to test the efficiency of the 1-octen-3-ol on rice samples contaminated with *S. oryzae*.

5.4 Application of radiations

Radiations such as ultraviolet, visible light, microwaves, infrared and radiofrequency waves can also be used as a tool for disinfestation. Some studies related to the application of these radiations have already been developed, obtaining very positive results. Duangkhamchan et al. (2017) studied the infrared heating method against *S. oryzae* in an egg stage, consisted of an electrical emitter with adjustable intensity by tuning the temperature. The results showed 100 % of insect mortality after two minutes of exposure, at all temperatures tested. (Pei et al., 2018) also studied the lethal effects of infrared radiation on *S. zeamais* (maize pest) and *Tribolium castaneum* in rice, concluding that heating the rice to 60 °C under infrared radiation of 2780 W/m² could be a feasible method for disinfestation. To treat the rice and insect samples, it was used a ceramic infrared drying device that consists of an infrared radiation emitter, a circulating fan, and a control panel. The mixture of rice and insects were heated to different temperatures under different infrared emitter temperatures. Then, the radiation of intensity of heated rice was measured and the authors concluded that with this treatment it was possible to achieve high insect mortality.

Other researchers have also been studied application of radiations as alternatives to the conventional treatments. Follett et al. (2013) studied an irradiation quarantine treatment for stored-products pests and the authors found out that this treatment has potential as a method to control rice weevil. They randomly selected 15 insect adults and placed on 500 g of rice in each of 20 plastic containers, which were treated with different radiation doses, and the number of live and dead adults was counted every week during five months. The conclusion was that 120 Gy radiation dose could be used for this pest control, and no further damage occurs to the rice grains.

Srivastava & Mishra (2022) investigated the application of microwave, ultraviolet, and vacuum, as well the combination of these three radiations in controlling adult stage of *R. dominica* in rice grains. The analysis was conducted in an equipment with microwave, ultraviolet irradiation, and temperature control. The process and status of the reactions in a container were observed via real time of the instant camera system. Their conclusions were that combination of the three treatments resulted better and leads to minimal changes in rice quality attributes.

The application of radiofrequency electromagnetic waves has been proposed to stored grain insect pest disinfestation. Radiofrequency technology is well developed for various applications such as pasteurization, being a form of rapid heating (similar to microwaves, just in different bandwidth). This method is a common nonchemical disinfestation process with effective and rapid action (Paul et al., 2020). The organic material like insect contains high moisture, shows high dielectric loss factors and heat can be transferred rapidly under electromagnetic field. When the energy is absorbed, the heat is generated rapidly in insects (Vearasilp et al., 2015) and radiofrequency technology can be applied to eliminate all stages of the insect lifecycle, from egg to adult. Vearasilp et al. (2015) report the construction of a simple radiofrequency heating pilot system whereby the rice simply falls through the radiofrequency field, reaches temperatures not in excess of 55 °C, and comes out completely clear of contamination of all forms of the weevil lifecycle after just 1-3 min of treatment. The quality of the rice before and after cooking was determined by instrumental methods and showed no significant difference to that of untreated rice. This system is environmentally friendly and safe for consumers, beyond that the authors concludes that this system is able to eliminate completely the rice weevils at any stage,

and no organoleptic assessment was verified. This technology appears very useful, as it could be applied to rice as in is transported into the storage silos and would then leave all rice free of infestation for storage.

6. Bio-packaging from rice by-products

The rice by-products (rice bran, rice husk/hull, and rice straw) are abundant wastes, partly used for low-value animal feed, which disposal is costly. However, added-value food bio-packaging, with biodegradable properties, may be produced from rice by-products. The resultant bio-packaging protects food against light, humidity, and other contaminants, and contributes to the increase in revenues of the rice industry. This waste reduction and valorisation into food bio-packaging, with a local production, are according to the Circular Economy basis.

6.1. Rice bran

Rice bran is the layer covering the rice grain, produced during the wet-milling process. Rice bran is rich in a huge number of bioactive compounds, very appealing to food ap-plications: phenolic and cinnamic acids, anthocyanins, flavonoids, steroidal compounds such as tocopherols, arabinoxylan and proteins (Friedman, 2013).

As far as bioplastics are concerned, rice bran has a relatively high content of valuable protein (about 10-15% (Amagliani et al., 2017; Fabian & Ju, 2011)) and of starch. Starch is a suitable and common biopolymer for packaging, its tensile properties are adequate for this application, and 50% of the commercial bio-packaging is prepared from starch (Marichelvam et al., 2019). Rice Bran-based bio-packaging typically comprises starch and protein, besides a plasticizer, commonly glycerol or sorbitol (Alonso-González et al., 2021, 2022a). Rice Bran-based bio-packaging has appealing thermoplastic properties, being produced by injection moulding, similarly to current petroleum-based packaging. Although rice bran oil is increasing its importance in cosmetic, food, and pharmaceutical applications, it should be removed during bio-packaging formulation, as it contributes negatively to the mechanical properties of bioplastics (Alonso-González et al., 2022b).

6.2. Rice husk/hull

Rice husk/hull is the layer covering the rice bran, produced during the wet-milling process. Rice husk/hull is composed mostly of lignin, hemicellulose, cellulose, and hydrated silica (Friedman, 2013), very hard materials. Rice husk/hull is commonly incinerated to produce energy and rice husk ash, a low-cost product with a high content of silica (83-90%). Rice husk ash is used in the production of silica gels, silicon chips, activated carbon and silica, lightweight construction materials, zeolites, and lithium batteries (Friedman, 2013).

Rice husk-based bioplastics are composed of mainly cellulose, and silica is used as fillers, with cellulose (Karaca et al., 2022). Starch-based bioplastics with silica as filler were demonstrated to be promising when compared to currently used plastics (Shafqat et al., 2021).

6.3. Rice straw

Rice straw is produced during harvesting and has a high content of cellulose. A bioplastic was produced based solely on the cellulose extracted from rice straw (Bilo et al., 2018). A composite bioplastic with starch as the matrix and the previously isolated cellulose nanocrystal CNC were formulated by casting with different starch-to-CNC ratios. The incorporation of cellulose nanocrystals in the bioplastic increased both its tensile strength and modulus but decreased its thermal stability (Agustin et al., 2014). Another composite bioplastic was proposed with cellulose from rice straw cellulose, and chitosan

and glycerol as additives. The increase of glycerol content led to a reduction in oil swelling, a more flexible (higher elongation at break), weaker (low tensile strength) (Inayati et al., 2019).

6. Conclusions

The conventional methods require the use of chemical insecticides, however, over the years some problems have arisen, such as potential risks to human health and the emergence of insecticide-resistant insects. Therefore, in view of this, some alternative treatments can be the used such as biopesticides, ozone gas, radiofrequency, microwaves, ultraviolet, vacuum, and infrared heating. The reported techniques are promising for the removing of insecticide residues in rice grains and some of them could be viable options for environmentally friendly insect management in stored rice grains. In future work, instead of using current petroleum-based packaging with the selected treatments for insect management, bio-packaging from rice by-products should be employed, following the principles of the Circular Economy.

Funding: The study was funded by project TRACE-RICE—Tracing rice and valorising side streams along Mediterranean blockchain, grant No. 1934, of the PRIMA Programme, supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation.

References

References must be numbered in order of appearance in the text (including citations in tables and legends) and listed individually at the end of the manuscript.

In the text, reference numbers should be placed in square brackets [ ] and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10), or [6] (pp. 101–105).

1. Qu, X., Kojima, D., Wu, L., & Ando, M. (2021). The losses in the rice harvest process: A review. In Sustainability (Switzerland) (Vol. 13, Issue 17). MDPI. https://doi.org/10.3390/su13179627
2. Mesterházy, ákos, Oláh, J., & Popp, J. (2020). Losses in the grain supply chain: Causes and solutions. In Sustainability (Switzerland) (Vol. 12, Issue 6). MDPI. https://doi.org/10.3390/su12062342
3. Zulaikha, S. A. S., & Yaakop, S. (2021). Effect on Sitophilus oryzae infestation on amylose content and weight loss of eight rice varieties. Pakistan Journal of Agricultural Sciences, 58(6), 1699–1703. https://doi.org/10.21162/PAKJAS/21.480
4. Müller, A., Nunes, M. T., Maldaner, V., Coradi, P. C., Moraes, R. S. de, Martens, S., Leal, A. F., Pereira, V. F., & Marin, C. K. (2022). Rice Drying, Storage and Processing: Effects of Post-Harvest Operations on Grain Quality. In Rice Science (Vol. 29, Issue 1, pp. 16–30). Elsevier B.V. https://doi.org/10.1016/j.rsci.2021.12.002
5. Yao, J., Wang, Z., Guo, L., Xu, X., Liu, L., Xu, L., Song, S., Xu, C., & Kuang, H. (2020). Advances in immunoassays for organophosphorus and pyrethroid pesticides. In TrAC - Trends in Analytical Chemistry (Vol. 131). Elsevier B.V. https://doi.org/10.1016/j.trac.2020.116022
6. Lee, S., Peterson, C. J., & Coats, J. R. (2003). Fumigation toxicity of monoterpenoids to several stored product insects. In Journal of Stored Products Research (Vol. 39).
7. Chou, C., Hadi, B. A. R., Chiba, S., Sato, I., Choi, I. R., & Tanaka, T. (2022). An entomopathogenic fungus and a natural extract benefit rice (Oryza sativa) by suppressing populations of insect pests while keeping high populations of their natural enemies. Biological Control, 165. https://doi.org/10.1016/j.biocontrol.2021.104793
8. Herrera, J. M., Gañán, N. A., Goñi, M. L., Zygadlo, J. A., & Martini, R. E. (2018). Active LDPE films loaded with biopesticides by supercritical CO2-assisted impregnation for stored grain protection. Food Packaging and Shelf Life, 18, 80–86. https://doi.org/10.1016/j.fpsl.2018.03.001
9. Zhao, L., Chen, L., Yuan, F., & Wang, L. (2022). Simulation Study of Rice Cleaning Based on DEM-CFD Coupling Method. Processes, 10(2), 281. https://doi.org/10.3390/pr10020281
10. Hagstrum, D. W., Phillips, T. W., & Cuperus, G. W. (2012). Stored product protection. Kansas State University.
11. Mancini, R., Carvalho, M. O., Timlick, B., Adler, C. Contribution for Integrated Management of Stored Rice Pests. IICT – Instituto de Investigação Científica Tropical, Palácio dos Condes da Calheta, Portugal, 2007, pp.

12. ISO 6339-4:2015
13. Howe, R. W., & Oxley, T. A. (n.d.). THE USE OF CARBON DIOXIDE PRODUCTION AS A MEASURE OF INFESTATION OF GRAIN BY INSECTS.
14. Xmgwei, H., Xianchang, T., & Jianchun', W. U. (n.d.). The comparison of five determination methods for hidden insect infestation.
15. Fleurat-Lessard, F. (2011). Monitoring insect pest populations in grain storage: The European context. In Stewart Postharvest Review (Vol. 7, Issue 3). https://doi.org/10.2212/spr.2011.3.4
16. Zhai, H. C., Zhang, S. B., Huang, S. X., & Cai, J. P. (2015). Prevention of toxigenic fungal growth in stored grains by carbon dioxide detection. Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 32(4), 596–603. https://doi.org/10.1080/19440049.2014.968221
17. Singh, C. B., & Fielke, J. M. (2017). IEEE Instrumentation & Measurement Magazine Recent Developments in Stored Grain Sensors, Monitoring and Management Technology.
18. Mankin, R., Hagstrum, D., Guo, M., Eliopoulos, P., & Njoroge, A. (2021). Automated applications of acoustics for stored product insect detection, monitoring, and management. Insects, 12(3). https://doi.org/10.3390/insects12030259
19. Banga, K. S., Kotwaliwale, N., Mohapatra, D., & Giri, S. K. (2018). Techniques for insect detection in stored food grains: An overview. In Food Control (Vol. 94, pp. 167–176). Elsevier Ltd. https://doi.org/10.1016/j.foodcont.2018.07.008
20. Santiago, R. M. C., Rabano, S. L., Billones, R. K. D., Calilung, E. J., Sybingco, E., & Dadios, E. P. (2017). Insect detection and monitoring in stored grains using MFCCs and artificial neural network. IEEE Xplore. https://doi.org/10.1109/TENCON.2017.8228290
21. Rabano, S., Cabatuan, M. K., Dadios, E. J. P., Calilung, E. J., Vicerra, R. R. P., Sybingco, E., & Regpala, E. R. (2018). Deep Transfer Learning based Acoustic Detection of Rice Weevils, *Sitophilus Oryzae* (L.) in Stored Grains. International Journal of Engineering & Technology, 7(4.16), 87–91. https://doi.org/10.14419/ijet.v7i4.16.21785
22. ISO
23. Doc chinês
24. Fu, L., Zhu, J., & Karimi-Maleh, H. (2021). An analytical method based on electrochemical sensor for the assessment of insect infestation in flour. Biosensors, 11(9). https://doi.org/10.3390/BIOS11090325
25. Germinara et al 2010
26. Shah, M. A., & Khan, A. A. (2014). Imaging techniques for the detection of stored product pests. In Applied Entomology and Zoology (Vol. 49, Issue 2, pp. 201–212). Springer Japan. https://doi.org/10.1007/s13355-014-0254-2
27. Srivastava, S., Mishra, G., & Mishra, H. N. (2020). Application of an expert system of X- ray micro computed tomography imaging for identification of Sitophilus oryzae infestation in stored rice grains. Pest Management Science, 76(3), 952–960. https://doi.org/10.1002/ps.5603
28. Boniecki, P., Koszela, K., Świerczyński, K., Skwarcz, J., Zaborowicz, M., & Przybył, J. (2020). Neural visual detection of grain weevil (Sitophilus granarius l.). Agriculture (Switzerland), 10(1). https://doi.org/10.3390/agriculture10010025
29. da Silva, C. B., Silva, A. A. N., Barroso, G., Yamamoto, P. T., Arthur, V., Toledo, C. F. M., & Mastrangelo, T. de A. (2021). Convolutional neural networks using enhanced radiographs for real-time detection of Sitophilus zeamais in maize grain. Foods, 10(4). https://doi.org/10.3390/foods10040879
30. Negi, A., Anandharaj, A., Kalakandan, S., & Rajamani, M. (2021). A molecular approach for the detection and quantification of Tribolium castaneum (Herbst) infestation in stored wheat flour. Food Technology and Biotechnology, 59(1), 112–121. https://doi.org/10.17113/ftb.59.01.21.6902
31. Nowaczyk, K., Obrepalska-Steplowska, A., Gawlak, M., Throne, J. E., Olejarski, P., & Nawrot, A. J. (2009). Molecular Techniques for Detection of Tribolium confusum Infestations in Stored Products. In J. Econ. Entomol (Vol. 102, Issue 4). https://academic.oup.com/jee/article/102/4/1691/2199296
32. Solà, M. (2016). Detection, identification, and quantification of insect infestation in stored products using PCR approaches. https://doi.org/10.1603/ice.2016.111999
33. Solà, M., Riudavets, J., & Agustí, N. (2018). Detection and identification of five common internal grain insect pests by multiplex PCR. Food Control, 84, 246–254. https://doi.org/10.1016/j.foodcont.2017.08.002
34.
35. Paul, A., Radhakrishnan, M., Anandakumar, S., Shanmugasundaram, S., & Anandharamakrishnan, C. (2020). Disinfestation techniques for major cereals: A status report. Comprehensive Reviews in Food Science and Food Safety, 19(3), 1125–1155. https://doi.org/10.1111/1541-4337.12555
36. Donahaye, E. J., Navarro, S., Bell, C., Jayas, D., & Noyes, R. (2007). DEVELOPING FUMIGATION PROTOCOLS TO MANAGE STRONGLY PHOSPHINE-RESISTANT RICE WEEVILS, SITOPHILUS ORYZAE (L.). FTIC Ltd. Publishing, Israel.
37. P Anankware, J. (2013). Triple-Layer Hermetic Storage: A Novel Approach Against *Prostephanus Truncatus* (Horn) Coleoptera: Bostrichidae) and *Sitophilus Zeamais* (Mot) (Coleoptera: Curculionidae). Entomology, Ornithology & Herpetology: Current Research, 02(03). https://doi.org/10.4172/2161-0983.1000113
38. Ali, M. P., Bari, M. N., Ahmed, N., Kabir, M. M. M., Afrin, S., Zaman, M. A. U., Haque, S. S., & Willers, J. L. (2017). Rice production without insecticide in smallholder farmer's field. Frontiers in Environmental Science, 5(MAY). https://doi.org/10.3389/fenvs.2017.00016

39. Li, B., Gao, K., Ren, H., & Tang, W. (2018). Molecular mechanisms governing plant responses to high temperatures. In Journal of Integrative Plant Biology (Vol. 60, Issue 9, pp. 757–779). Blackwell Publishing Ltd. https://doi.org/10.1111/jipb.12701
40. Champagne, E. T. (2004). Rice: chemistry and technology (No. Ed. 3). American Association of Cereal Chemists.
41. Fields, P. G. (1992). THE CONTROL OF STORED-PRODUCT INSECTS AND MITES WITH EXTREME TEMPERATURES*. In J. stored Prod. Res (Vol. 28, Issue 2).
42. Bhattacharya, K. R. (2011). Rice quality: A guide to rice properties and analysis. Elsevier.
43. Maier, D.E., Navarro, S., (2002). Chilling of grain by refrigerated air. In: Navarro, S., Noyes, R. (Eds). The Mechanics and Physics of Modern Aeration Management. CRC PRESS, pp. 491-560.
44. Maier, D. E. (1994). Chilled aeration and storage of US crops-a review. In Proceedings. 6th International Working Conference on Stored-Product Protection (Vol. 1, pp. 300-311). Wallingford: CAB International.
45. U.S. Congress (1989). Enhancing the Quality of U.S. Grain for International Trade. Office of Technology Assessment, Washington, DC.
46. Katta, Y. M., Kamara, M. M., Abd El-Aty, M. S., Elgamal, W. H., Soleiman, R. M., Mousa, K. M., & Ueno, T. (2019). Effect of storage temperature on storage efficacy, germination and physical characters of some paddy rice cultivars during different storage periods. Journal of the Faculty of Agriculture, Kyushu University, 64(1), 61–69. https://doi.org/10.5109/2232279
47. Morales Quiros, A. (2017). Evaluation of ambient and chilled aeration strategies to maintain the quality of stored grain in tropical climates and during summer in temperate climates (Doctoral dissertation, Kansas State University).
48. Lazzari, F. A., Lazzari, S. M. N., Karkle, A. F., Lorini, B., Bacaltchuk, H., Beckel, D., & de OF, B. (1994). Artificial cooling to control coleopterans in paddy rice stored in metallic silo. In Proceedings of the 9th International Working Conference on Stored-Product Protection (pp. 15-18).
49. Lazzari, F. A., Lazzari, S. M. N., & Lazzari, F. N. (2010). Environmentally friendly technologies to maintain stored paddy rice quality. Julius-Kühn-Archiv, (425), 710-715.
50. Rees, D. P. (2004). Insects of stored products. London, England: Manson Pub.
51. Carvalho, M. O., Fradinho, P., Martins, M. J., Magro, A., Raymundo, A., & de Sousa, I. (2019). Paddy rice stored under hermetic conditions: The effect of relative humidity, temperature and storage time in suppressing *Sitophilus zeamais* and impact on rice quality. Journal of Stored Products Research, 80, 21–27. https://doi.org/10.1016/j.jspr.2018.11.002
52. Covele, G., Gulube, A., Tivana, L., Ribeiro-Barros, A. I., Carvalho, M. O., Ndayiragije, A., & Nguenha, R. (2020). Effectiveness of hermetic containers in controlling paddy rice (*Oryza sativa* L.) storage insect pests. Journal of Stored Products Research, 89. https://doi.org/10.1016/j.jspr.2020.101710
53. Guenha, R., Salvador, B. das V., Rickman, J., Goulao, L. F., Muocha, I. M., & Carvalho, M. O. (2014). Hermetic storage with plastic sealing to reduce insect infestation and secure paddy seed quality: A powerful strategy for rice farmers in Mozambique. Journal of Stored Products Research, 59, 275–281. https://doi.org/10.1016/j.jspr.2014.06.007
54. Martin, D. T., Baributsa, D., Huesing, J. E., Williams, S. B., & Murdock, L. L. (2015). PICS bags protect wheat grain, Triticum aestivum (L.), against rice weevil, Sitophilus oryzae (L.) (Coleoptera: Curculionidae). Journal of Stored Products Research, 63, 22–30. https://doi.org/10.1016/j.jspr.2015.05.001
55. Carvalho, M. O., Pires, I., Barbosa, A., Barros, G., Riudavets, J., Garcia, A. C., Brites, C., & Navarro, S. (2012). The use of modified atmospheres to control Sitophilus zeamais and Sitophilus oryzae on stored rice in Portugal. Journal of Stored Products Research, 50, 49–56. https://doi.org/10.1016/j.jspr.2012.05.001
56. Savi, G. D., Gomes, T., Canever, S. B., Feltrin, A. C., Piacentini, K. C., Scussel, R., Oliveira, D., Machado-de-Ávila, R. A., Cargnin, M., & Angioletto, E. (2020). Application of ozone on rice storage: A mathematical modeling of the ozone spread, effects in the decontamination of filamentous fungi and quality attributes. Journal of Stored Products Research, 87. https://doi.org/10.1016/j.jspr.2020.101605
57. Amoah, B. A., & Mahroof, R. M. (2019). Ozone as a Potential Fumigant Alternative for the Management of Sitophilus oryzae (Coleoptera: Curculionidae) in Wheat. Journal of Economic Entomology, 112(4), 1953–1963. https://doi.org/10.1093/jee/toz071
58.
59. de Ávila, M. B. R., Faroni, L. R. A., Heleno, F. F., de Queiroz, M. E. L. R., & Costa, L. P. (2017). Ozone as degradation agent of pesticide residues in stored rice grains. Journal of Food Science and Technology, 54(12), 4092–4099. https://doi.org/10.1007/s13197-017-2884-1
60. Debnath, N., Das, S., Seth, D., Chandra, R., Bhattacharya, S. Ch., & Goswami, A. (2010). Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). Journal of Pest Science, 84(1), 99–105. https://doi.org/10.1007/s10340-010-0332-3
61. Kar, S., Nayak, R. N., Sahoo, N. R., Bakhara, C. K., Panda, M. K., Pal, U. S., & Bal, L. M. (2021). Rice weevil management through application of silica nano particle and physico-chemical and cooking characterization of the treated rice. Journal of Stored Products Research, 94, 101892. https://doi.org/10.1016/j.jspr.2021.101892
62. Chang, Y., Lee, S. H., Na, J. H., Chang, P. S., & Han, J. (2017). Protection of Grain Products from Sitophilus oryzae (L.) Contamination by Anti-Insect Pest Repellent Sachet Containing Allyl Mercaptan Microcapsule. Journal of Food Science, 82(11), 2634–2642. https://doi.org/10.1111/1750-3841.13931
63. Follett, P. A., Rivera-Leong, K., & Myers, R. (2014). Rice weevil response to basil oil fumigation. Journal of Asia-Pacific Entomology, 17(2), 119–121. https://doi.org/10.1016/j.aspen.2013.11.008
64. Shi, W., Yan, R., & Huang, L. (2021). Preparation and insecticidal performance of sustained-release cinnamon essential oil microemulsion. Journal of the Science of Food and Agriculture. https://doi.org/10.1002/jsfa.11472

65. Guettal, S., Tine, S., Hamaidia, K., Tine-Djebbar, F., & Soltani, N. (2020). Effect of *Citrus limonum* essential oil against granary weevil, *Sitophilus granarius* and its chemical composition, biological activities and energy reserves. International Journal of Tropical Insect Science, 41(2), 1531–1541. https://doi.org/10.1007/s42690-020-00353-y
66. Mishra, B. B., Tripathi, S. P., & Tripathi, C. P. M. (2013). Bioactivity of two plant derived essential oils against the rice weevils *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Proceedings of the National Academy of Sciences India Section B - Biological Sciences, 83(2), 171–175. https://doi.org/10.1007/s40011-012-0123-0
67. Bhavya, M. L., Chandu, A. G. S., & Devi, S. S. (2018). *Ocimum tenuiflorum* oil, a potential insecticide against rice weevil with anti-acetylcholinesterase activity. Industrial Crops and Products, 126, 434–439. https://doi.org/10.1016/j.indcrop.2018.10.043
68. Al-Harbi, N. A., Al Attar, N. M., Hikal, D. M., Mohamed, S. E., Abdel Latef, A. A. H., Ibrahim, A. A., & Abdein, M. A. (2021). Evaluation of Insecticidal Effects of Plants Essential Oils Extracted from Basil, Black Seeds and Lavender against *Sitophilus oryzae*. Plants, 10(5), 829. https://doi.org/10.3390/plants10050829
69. Goñi, M. L., Gañán, N. A., Herrera, J. M., Strumia, M. C., Andreatta, A. E., & Martini, R. E. (2017). Supercritical CO2iof LDPE films with terpene ketones as biopesticides against corn weevil (Sitophilus zeamais). Journal of Supercritical Fluids, 122, 18–26. https://doi.org/10.1016/j.supflu.2016.11.017
70. Codex Alimentarius 2017
71. Soujanya, P. L.; Sekhar, J. C.; Suby, S. B.; Rakshit, S.; Srival, G. Biopesticide treated double layered bags: novel method of application of botanicals for Sitophilus oryzae L. management in stored maize. Maydica, [S.l.], v. 63, n. 1, p. 6, apr. 2018. ISSN 2279-8013. Available at: <https://journals-crea.4science.it/index.php/maydica/article/view/1600/1100>. Date accessed: 19 sep. 2022.
72. Silva, M. F., Maciel, V. B. V., Noletto, A. P. R., Venturini, A. C., Carvalho, R. A., & Yoshida, C. M. P. (2022). Chitosan active coating on paperboard surface forming an anti-insect grain-based food packaging. Packaging Technology and Science, 35(4), 361–372. https://doi.org/10.1002/pts.2634
73. Duangkhamchan, W., Phomphai, A., Wanna, R., Wiset, L., Laohavanich, J., Ronsse, F., & Pieters, J. G. (2016). Infrared Heating as a Disinfestation Method Against Sitophilus oryzae and Its Effect on Textural and Cooking Properties of Milled Rice. Food and Bioprocess Technology, 10(2), 284–295. https://doi.org/10.1007/s11947-016-1813-z
74. Pei, Y., Tao, T., Yang, G., Wang, Y., Yan, W., & Ding, C. (2018). Lethal effects and mechanism of infrared radiation on *Sitophilus zeamais* and *Tribolium castaneum* in rough rice. Food Control, 88, 149–158. https://doi.org/10.1016/j.foodcont.2018.01.012
75. Follett, P. A., Snook, K., Janson, A., Antonio, B., Haruki, A., Okamura, M., & Bisel, J. (2013). Irradiation quarantine treatment for control of *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice. Journal of Stored Products Research, 52, 63–67. https://doi.org/10.1016/j.jspr.2012.09.004
76. Srivastava, S., & Mishra, H. N. (2022). Disinfestation of Rhyzopertha dominica Coleoptera: bostrichidae by combinational approach of microwave ultraviolet and vacuum assisted process in stored rice grains. International Journal of Tropical Insect Science, 42(2), 1535–1542. https://doi.org/10.1007/s42690-021-00672-8
77. Vearasilp, S., Thanapornpoonpong, S., Krittigamas, N., Suriyong, S., Akaranuchat, P., & von Hörsten, D. (2015). Vertical Operating Prototype Development Supported Radio Frequency Heating System in Controlling Rice Weevil in Milled Rice. Agriculture and Agricultural Science Procedia, 5, 184–192. https://doi.org/10.1016/j.aaspro.2015.08.028
78. Friedman, M. (2013). Rice brans, rice bran oils, and rice hulls: Composition, food and industrial uses, and bioactivities in humans, animals, and cells. In Journal of Agricultural and Food Chemistry (Vol. 61, Issue 45, pp. 10626–10641). https://doi.org/10.1021/jf403635v
79. Amagliani, L., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2017). The composition, extraction, functionality and applications of rice proteins: A review. In Trends in Food Science and Technology (Vol. 64, pp. 1–12). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2017.01.008
80. Fabian, C., & Ju, Y.-H. (2011). A Review on Rice Bran Protein: Its Properties and Extraction Methods. Critical Reviews in Food Science and Nutrition, 51(9), 816–827. https://doi.org/10.1080/10408398.2010.482678
81. Marichelvam, M. K., Jawaid, M., & Asim, M. (2019). Corn and Rice Starch-Based Bio-Plastics as Alternative Packaging Materials. Fibers, 7(4), 32. https://doi.org/10.3390/fib7040032 a
82. Alonso-González, M., Felix, M., Guerrero, A., & Romero, A. (2021). Effects of mould temperature on rice bran-based bioplastics obtained by injection moulding. Polymers, 13(3), 1–12. https://doi.org/10.3390/polym13030398
83. Alonso-González, M., Felix, M., & Romero, A. (2022a). Influence of the plasticizer on rice bran-based eco-friendly bioplastics obtained by injection moulding. Industrial Crops and Products, 180. https://doi.org/10.1016/j.indcrop.2022.114767
84. Alonso-González, M., Felix, M., & Romero, A. (2022b). Rice bran-based bioplastics: Effects of biopolymer fractions on their mechanical, functional and microstructural properties. Polymers, 14(1). https://doi.org/10.3390/polym14010100
85. Karaca, A. E., Özel, C., Özarslan, A. C., & Yücel, S. (2022). The simultaneous extraction of cellulose fiber and crystal biogenic silica from the same rice husk and evaluation in cellulose-based composite bioplastic films. Polymer Composites. https://doi.org/10.1002/pc.26729
86. Shafqat, A., Tahir, A., Ullah Khan, W., Mahmood, A., Abbasi, G. H., & Khan, W. U. (2021). PRODUCTION AND CHARACTERIZATION OF RICE STARCH AND CORN STARCH BASED BIODEGRADABLE BIOPLASTIC USING VARIOUS PLASTICIZERS AND NATURAL REINFORCING FILLERS. In CELLULOSE CHEMISTRY AND TECHNOLOGY Cellulose Chem Technol (Vol. 55, Issue 8).
87. Bilo, F., Pandini, S., Sartore, L., Depero, L. E., Gargiulo, G., Bonassi, A., Federici, S., & Bontempi, E. (2018). A sustainable bioplastic obtained from rice straw. Journal of Cleaner Production, 200, 357–368. https://doi.org/10.1016/j.jclepro.2018.07.252

88. Agustin, M. B., Ahmmad, B., Alonzo, S. M. M., & Patriana, F. M. (2014). Bioplastic based on starch and cellulose nanocrystals from rice straw. Journal of Reinforced Plastics and Composites, 33(24), 2205–2213. https://doi.org/10.1177/0731684414558325
89. Inayati, Pamungkas, D. J., & Matovanni, M. P. N. (2019). Effect of glycerol concentration on mechanical characteristics of biodegradable plastic from rice straw cellulose. AIP Conference Proceedings, 2097. https://doi.org/10.1063/1.5098285

toxins

MDPI

Review

Mycotoxins Contamination in Rice: Analytical Methods, Occurrence and Detoxification Strategies

Ana Rita Santos [1], Filipa Carreiró [1,2], Andreia Freitas [2,3], Sílvia Barros [2], Carla Brites [2,4], Fernando Ramos [1,3] and Ana Sanches Silva [1,2,5,6,*]

[1] Faculty of Pharmacy, University of Coimbra, Polo III, Azinhaga de Stª Comba, 3000-548 Coimbra, Portugal
[2] National Institute for Agricultural and Veterinary Research (INIAV), I.P., Av. da República, 2780-157 Oeiras, Portugal
[3] Associated Laboratory for Green Chemistry of the Network of Chemistry and Technology, REQUIMTE/LAQV, R. D. Manuel II, Apartado 55142, 4051-401 Porto, Portugal
[4] GREEN-IT Bioresources for Sustainability, ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal
[5] Centre for Animal Science Studies (CECA), ICETA, University of Porto, 4501-401 Porto, Portugal
[6] Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal
* Correspondence: ana.silva@iniav.pt or anateress@gmail.com

Abstract: The prevalence of mycotoxins in the environment is associated with potential crop contamination, which results in an unavoidable increase in human exposure. Rice, being the second most consumed cereal worldwide, constitutes an important source of potential contamination by mycotoxins. Due to the increasing number of notifications reported, and the occurrence of mycotoxins at levels above the legislated limits, this work intends to compile the most relevant studies and review the main methods used in the detection and quantification of these compounds in rice. The aflatoxins and ochratoxin A are the predominant mycotoxins detected in rice grain and these data reveal the importance of adopting safety storage practices that prevent the growth of producing fungi from the Aspergillus genus along all the rice chain. Immunoaffinity columns (IAC) and QuECHERS are the preferred methods for extraction and purification and HPLC-MS/MS is preferred for quantification purposes. Further investigation is still required to establish the real exposition of these contaminants, as well as the consequences and possible synergistic effects due to the co-occurrence of mycotoxins and also for emergent and masked mycotoxins.

Keywords: Co-occurrence; HPLC-MS; Mitigation; Mycotoxins; QuEChERS; Rice

Key Contribution: Rice is one of the most consumed cereals worldwide, resulting in a large exposure to its potential contaminants. IAC and QuECHERS are the preferred methods for extraction and purification of mycotoxins in rice, also HPLC-MS/MS is preferred for quantification of mycotoxins in rice.

Citation: Santos A.R.; Carreiró F.; Freitas A.; Barros S.; Brites C.; Ramos F. and Silva A.S. Mycotoxins Contamination in Rice: Analytical Methods, Occurrence and Detoxification Strategies. *Toxins* **2022**, *14*, 647. https://doi.org/10.3390/toxins14090647

Received: 23 July 2022
Accepted: 9 September 2022
Published: 19 September 2022

1. Introduction

Mycotoxins are secondary products resulting from toxigenic fungal metabolism. They consist of low molecular weight metabolites and are mostly produced by the genus *Aspergillus*, *Fusarium*, and *Penicillium* [1]. Over 400 types of mycotoxins have been identified, but attention is mainly given to those with the greatest public health relevance, such as aflatoxins (AFs), ochratoxin A (OTA), fumonisins (FUMs), trichothecenes (TCs) and zearalenone (ZEA) [1,2]. Their structural diversity results in different chemical and physicochemical properties, and they are associated with the development of acute and chronic problems such as carcinogenicity, teratogenicity, mutagenicity, and hepatotoxicity [1,3].

Due to their worldwide prevalence and their association with health disorders, my-

cotoxins have been recognized as a major health and economic issue [4]. In fact, these toxins are considered by European Food Safety Authority (EFSA) as a threat and are one of the most reported hazards on RASFF (Rapid Alert System for Food and Feed) [5].

The European Commission (EC) has established a regulation where the maximum levels allowed for some mycotoxins are established, but many studies have reported cases where those limits are exceeded [6]. Therefore, and due to climate change, strict control is required, as well as the development and validation of suitable analytical methods [7].

It is almost impossible to avoid the presence of mycotoxins in the food chain, but their levels can be controlled by the implementation of good agriculture practices and decontamination processes [1].

The present review comprises a review of the most commonly found mycotoxins in rice and the main methods used for their extraction, detection, and quantification, as well as the techniques used in decontamination processes.

2. Mycotoxins

Over 400 mycotoxins have been identified to date, but only a few represent known concerns to human health, including AFs, OTA, DON, T-2/HT-2 toxins, FUMs, and ZEA [1].

Aflatoxins are a family of mycotoxins produced by a fungus of the genus *Aspergillus* (mainly *A. flavus* and *A. parasiticus*), which can be found in rice [2]. Among all classes of mycotoxins, aflatoxins are thought to be the most toxic, and the greatest concern, not only at economic level (mainly in the United States and European Union) but also in health terms, contributing to hundreds of hepatocellular carcinoma (HCC) cases every year in developing countries [8,9].

The most relevant aflatoxins are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), and aflatoxin M1 (AFM1), with aflatoxin B1 being the most commonly occurring and toxic one [10].

Ochratoxins are produced by *Aspergillus* or *Penicillium*, mainly *A. ochraceus* and *P. verrucosum*, under variable environmental conditions. Ochratoxin A is known to be the most toxic and prevalent in this class [10,11]. Ochratoxins are found to be stable in acidic conditions and elevated temperatures. This thermal resistance makes them difficult to eliminate under normal cooking conditions [11]. OTA is considerably prevalent in cereals [12].

Fumonisins are fungal toxins produced by *Fusarium* spp. (mainly *F.verticilloides* and *F. proliferatum*), found most frequently in maize and cereals. This class of mycotoxins is known to be non-fluorescent and hydrophilic, unlike other classes, that can be completely dissolved in organic solvents [11]. There are more than 28 known fumonisins, divided into four main groups: A, B, C, and P. The fumonisins B group is the most frequent in nature and comprises fumonisin B1 (FB1), fumonisin B2 (FB2), and fumonisin B3 (FB3), with FB1 being the most toxic and frequent member of the family (70-80% of all fumonisins) [13].

TCs are a group of mycotoxins mainly produced by fungal species of Fusarium spp. This family is organized into four groups: the trichothecenes A, B, C, and D, each with structurally related toxins. Types A and B TCs are the most frequent in the group [14]. Type A TCs are the most toxic and include T-2 and HT-2 toxins. These toxins are mainly produced by *F. lansehtiae*, *F. sporotrichioides, F.poae,* and *F.acumminatum*, and have been detected in many food matrices including barley, oat, wheat, rice, and maize [13]. Type B TCs include nivalenol and deoxynivalenol (DON), with the last one being the most frequent, although less toxic, of the group. DON is predominantly produced by *F. culmorum* and *F. graminearum* and can be found in cereal and cereal-based products, widely distributed [14]. Of all classes of mycotoxins, trichothecenes are the most structurally diversified, and mainly contaminate cereals, such as maize, rice, oats, wheat, and barley [15].

Zearalenone (ZEA) is a macrocyclic lactone produced by multiple species of *Fusarium*, mainly *F. graminearum*, *F. sporotrichioides* and *F. semitectum.* It is usually associated with maize crops, but it can also be found in other cereals such as wheat, barley, rice, and oats. This toxin tends to appear mostly in temperate and warm countries with high humidity levels [13]. ZEA´s contamination usually occurs concurrently with DON or, less frequently, with aflatoxins. This mycotoxin can be partially eliminated under elevated temperatures but is stable under normal cooking conditions [16].

2.1. Emerging Mycotoxins

Emerging mycotoxins can be defined as a group of mycotoxins that has not been routinely determined or legislatively regulated, but the evidence of their incidence has been rapidly increasing in the last few decades [16].

Enniatins (ENNs) and beauvericin (BEA) are structurally related mycotoxins that belong to this class, produced by many filamentous fungi. ENNs are mainly produced by *Fusarium* spp, *Alternaria* spp, *Halosapheia* spp, and *Verticillum* spp, while BEA is mostly produced by *Beauveria* spp., *Paecilomyces* spp., *Polyporus* spp., and *Fusarium* spp [17,18]. These emerging mycotoxins have been reported in several matrices in recent publications, but their toxic effects have not yet been well established. The main source of contamination of these mycotoxins are cereals (including maize, wheat, barley, and rice), not only for being ideal matrices for fungal growth but also because of their great consumption among the population [17].

To date, 29 enniatin analogs have been reported, with enniatin A (ENN A), A_1 (ENN A_1), B (ENN B), B_1 (ENN B_1), and B_4 (ENN B_4) being the most prevalent, but there have also been found lower amounts of enniatins C, D, E, and F. Their structural differences are responsible for the distinct bioactivities of these analogs [18].

Studies have shown that emerging mycotoxins are prevalent worldwide and are able to co-occur with other classes of mycotoxins. Therefore, they might be a hazard to human and animal health. There have been no reports found on mycotoxicosis caused by BEA and ENNs, although some studies have described possible risks associated with their ingestion due to their ionophoric properties. Further investigation needs to be done in order to evaluate their health risk and eventually come up with regulatory levels [17,18].

Moniliformin (MON) and sterigmatocystin (STC) are also emerging mycotoxins that have also already been reported in rice. STC has the particularity of being a precursor of AFB1, and so they share a similar mechanism of toxicity, by forming Deoxyribonucleic acid (DNA) adducts and generating reactive oxygen species (ROS). This can lead to false negatives or underdetermination of AFB1 since STC can be later converted into its successor, considered by many authors as the most toxic and concerning mycotoxin [16]. *Alternaria* toxins, such as alternariol and tenuazonic acid, and citrinin (CIT) are other examples of emerging mycotoxins, mostly detected in fruits and vegetables [16].

2.2. Masked Mycotoxins

Masked mycotoxins are produced by plant enzymes involved in detoxification processes or during food processing through conjugation with polar substances such as glucose, sulfate, and amino acids. This structure modification leads to difficulty in their detection by conventional analytical methods [17,19].

Deoxynivalenol-3-glucoside (DON-3G) and ZEA-14-glucoside (ZEA-14G) are among the most commonly detected conjugates. Those conjugations are an attempt of the plants to make the compounds more soluble in water for faster elimination, and they usually exhibit lower toxicity in comparison with parent forms [20].

When metabolized, the masked mycotoxins suffer hydrolyzation and release the original mycotoxin. This can also happen during processing and constitutes a concern, because masked mycotoxins are not being accounted for by analytical methods and a

food commodity that was judged as compliant might become non-compliant at a later stage, because of the release of the mycotoxin [19].

2.3. Co-Occurrence

Co-occurrence consists of the occurrence of multiple mycotoxins within the same food matrix [2]. Multiple exposures are very frequent, being even more common than the presence of a single mycotoxin [10]. Although there is still a lot to know, the co-occurrence of mycotoxins may result in additive or synergistic effects, increasing the toxicity of the contaminated material [2].

In rice, the occurrence of different mycotoxins and their metabolites is unavoidable due to the simultaneous infection with multiple fungi, that are toxigenic, i.e. they are able to produce multiple mycotoxins [21].

AFB1 and AFB2 are the most frequently documented as co-occurring mycotoxins, but it has also been reported that the co-occurrence of mycotoxins is produced by different fungi species [21]. It has been described in several studies the combined effects of mycotoxins; however, it is still unknown the nature of the observed effects, the relative potencies of each mycotoxin, and the way those interactions could enhance their respective toxic effects [10,17].

2.4. Mycotoxins-Producing Fungi

Mycotoxin-producing fungi mostly belonging to the genera *Aspergillus*, *Fusarium* and *Penicillium* are among the organisms able to contaminate rice [15].

Fungi growing conditions are dependent on many factors, such as the presence of fungal inoculum on susceptible crops, fertilization balance, insect damage, inadequate storage conditions, temperature, humidity, water activity (a_w), pH and nutritional composition of the food product, and so their relevance is different around the world [21,22]. Weather variables are the leading factors contributing to mycotoxin occurrence, but the cropping system used is a powerful tool for farmers to mitigate grain contamination [23].

Even inside the same genera, different species may grow during different stages of production [15].

Aspergillus grows predominately in tropical countries, with high temperatures paired with high values of RH and a_w. For example, rice in tropical Asia is mostly contaminated with *Aspergillus* fungi (such as *A. flavus* and *A. ochraceus*) because of the conditions during pre-harvest (improved crop management and agronomic practices, control of insects that favor fungal infection, host plant resistance, and biological control, such Afla-Guard® GR from Syngenta ® (Iowa, United States) that can be used in maize, which active ingredient is a nontoxigenic strain of *A. flavus* that acts by competitively displacing toxigenic, aflatoxin-producing strains, something similar should be specific to rice), harvest and postharvest stages [21,22]. Despite being difficult to predict the occurrence of fungal diseases and toxin contamination in food grains predictive models can be used and most publications on predictive mycology have just come up during the last decade [22,24,25]. A model is a simplified representation of a system, which is a limited part of reality and contains interrelated elements and attempts to summarize the main processes, put forward hypotheses, and verify their coherence and consequences [22]. Prediction models have been developed, based on several impact factors that might influence mycotoxins occurrence. Especially, in terms of the effect that climate change may have nowadays in the future, those models have been used to calculate the associated risks for human and animal health and with these models, the final levels of mold or mycotoxins contamination may be predicted (a useful tool for the food industry) [24,25].

Fusarium spp. grows under high temperatures and moisture and is the major cause of a decline in rice quality during cultivation due to environmental conditions [15]. *Penicillium* spp. is not found in the field during the growing period, and their contamination is usually associated with rice storage conditions [15].

It is well known that not all fungi are threatening and not all their secondary metabolites are toxic. Mycotoxins' toxicity depends not only on their producer but also on their interaction with each other and with other microorganisms, on the edaphoclimatic conditions, and on the system of farm management (organic versus conventional) to which they are submitted [21]. Moreover, fungal contamination of certain food matrices, is not a synonym of contamination with mycotoxins, since fungi only produce these metabolites under specific circumstances as a strategic defensive mechanism. Therefore, the production of mycotoxins might not be associated with the presence of the fungal itself, but with the presence of other fungi or microbes, or even with the fluctuation of the environmental conditions (such as water availability and temperature) [26].

2.5. Factors Associated with Rice Contamination by Mycotoxins

Food contamination by mycotoxins is dependent on the presence of fungi, the application of unsuitable agricultural practices and the conditions of harvesting, and storage. Since most mycotoxins are thermostable and consequently able to persist under food processing and cooking temperatures, the key to their absence must be based on the prevention of their occurrence [27].

Mycotoxins' contamination may occur in different stages, from pre-harvest to post-harvest steps, during processing, packaging, distribution, or even storage. The rice grain is harvested with husk and their physical structure exerts a protective effect against field mycotoxin contaminations. Usually, mycotoxins' contamination in rice grain is associated with fungal growth due to improper storage conditions [28].

Despite the protective grain layers, paddy rice is susceptible to contamination after harvesting, since almost of worldwide rice production is harvested in subtropical environments (under warm and humid conditions), and then stored for large amounts of time before its consumption. When stored under inappropriate conditions it constitutes a great substrate for fungal growth. According to the Food and Agriculture Organization (FAO), every year around 15% of the rice harvest is lost due to fungal growth and mycotoxin contamination [29,30].

Rice crop development is strongly dependent on temperature since it has a great impact on plant photosynthesis, which when submitted to temperature stress, suffers a reduction in physiological activity. Therefore, climate change may have a substantial impact on rice grain production. Along with temperature increase, projections point to a decrease in precipitation along the Mediterranean basin area, which should have a negative impact on this crop, since it is very dependent on water supply [31,32].

Climate changes are also increasing mycotoxins' contamination. Earth temperature is expected to increase 1.5 to 4.5 ºC until the end of the 21st century. Global warming boosts water evaporation from the surface, which results in an increase in moisture within the atmosphere. Consequently, an increase in the fungal population and mycotoxins' occurrence is expected since temperature and humidity are key factors for their growth [32].

2.6. Toxicity and Mechanisms of Action of Mycotoxins

Mycotoxins' contamination is associated with multiple risks to human health due to their toxicity, in particular their carcinogenicity. In order to avoid these risks, taking into account epidemiological, experimental, and mechanism studies, the International Agency for Research on Cancer (IARC) has come up with a scale of hazard assessment of mycotoxins in human health [33].

Mycotoxin ingestion can result in both acute and chronic toxicity. Acute toxicity is associated with a rapid toxic response, while chronic toxicity is a result of low-dose exposure over a long period. Although chronic toxicosis has been found to be a global problem, acute toxicosis is more common in developing countries, particularly in Africa [8,33].

Aflatoxins have carcinogenic, mutagenic, hepatotoxic, teratogenic, and immunosuppressive effects, with the liver being the most affected organ. AFB1 is the most toxic of all aflatoxins, with AFB2, AFG1, and AFG2 having, respectively, 50, 20, and 10% of its toxigenic power [17]. Aflatoxins have been classified by IARC as a Group 1 carcinogen, due to the high risk of development of HCC after chronic exposure. AFM1 is a result of AFB1's biotransformation and has been classified as a Group 2B (possibly carcinogenic to humans). In humans, acute aflatoxicosis usually results in abdominal pain, vomiting, pulmonary and cerebral edema, coma, convulsions, or even death [33,34].

After being ingested, aflatoxins are biotransformed in the liver by a family of enzymes called CYPP450. These are responsible for turning AFB1 into its carcinogenic form: AFB-8,9-epoxide. This metabolite is able to form adducts with cellular macromolecules, such as DNA, which results in a modification of its structure and biological activity, and therefore in the carcinogenic and mutagenic effects of the toxin. A mutation of gene p53 seems to be the base of the association between aflatoxins and HCC, and this type of cancer is found to be more prevalent in regions with high consumption of aflatoxins [35].

In countries with a high rate of hepatitis B virus (HBV), exposure to AFB1 may constitute an even bigger issue, since the risk of liver cancer development after exposure to aflatoxins in HBV-positive people is about 30 times greater than in HBV-negative people [27].

Ochratoxin A is a fat-soluble mycotoxin that has been classified by IARC as Group 2B (possible human carcinogen) and is associated with immunotoxicity, neurotoxicity, genotoxicity, and embryotoxicity in both humans and animals [34,36]. Its toxicity seems to be related to its structural similarity with phenylalanine, an essential amino acid. OTA inhibits proper protein synthesis in the kidney and liver, by interfering with phenylalanine hydroxylase. It also seems to interfere with DNA and RNA synthesis [36].

Fumonisins are classified by IARC as belonging to Group 2B (possibly carcinogenic to humans) and seem to be associated with esophageal tumors and liver toxicity [34,37]. FB1 is found to be the most abundant and toxic of the group, followed by FB2 and FB3. Recent studies have been focusing on FUM's mechanism of action, and their similarity to sphinganine and sphingosine has come to attention with their possible role in the inhibition of sphingolipids biosynthesis. These sphingolipids are allocated on the membrane of eukaryotic cells and are responsible for the formation of secondary messengers, involved in the regulation of several cellular processes such as gene expression and protein activation/deactivation. By disrupting these mechanisms, this class of mycotoxins might contribute to many effects at a cellular level such as apoptosis induction and carcinogenic effects [38].

Some studies have correlated the levels of FBs in food with the development of esophageal cancer in humans. Moreover, they also seem to be associated with brain and spinal cord neural tube defects, when ingested at high levels during pregnancy [38].

ZEA is frequently described as an estrogenic mycotoxin due to its structural similarity to estrogens. Because of that, ZEA and its metabolites are able to bind competitively to estrogen receptors, activate the estrogen gene, and induce reproductive disorders. Long-time exposure to ZEA has also been shown to be associated with liver lesions and HCC development in the worst cases [39]. ZEA is associated with cytotoxic, hematologic, genotoxic, hepatotoxic, and immunotoxic effects, and has been classified by IARC as a group 3 carcinogenic (not classified as human carcinogenic) due to reduced evidence in experimental animals and inadequate evidence in humans [4,34].

Trichothecenes can easily penetrate cell membranes and react with cellular organelles and nucleic acids, which justifies their high toxicity. The major mechanism described consists of the inhibition of ribosomal protein synthesis, followed by disruption of DNA and RNA synthesis [40].

DON has been found to be immunosuppressant and genotoxic, but due to a lack of evidence of carcinogenicity, was classified by IARC as group 3 carcinogenic (not classi-

fied as human carcinogenic) [4,34,37]. Nausea, vomiting, diarrhea, dizziness, and fever are some of the reported effects of human exposure to DON-contaminated grains [41].

T-2 toxins have also been classified as group 3 by IARC, and along with HT-2 toxins, have been associated with a reduction in body weight, liver and kidney toxicity, immunotoxicity, neurotoxicity, and haematotoxic effects [4,34].

2.7. Mycotoxins Legislation with Special Focus at EU Level

Due to the global toxic effects of mycotoxins, a vast number of governmental authorities, including the Food and Drug Administration (FDA), World Health Organization (WHO), EFSA, and FAO, are paying attention and setting maximum levels for mycotoxins in foodstuffs, in order to protect human health [9]. The availability of toxicological information and dietary exposure, along with the distribution of mycotoxins and the available analytical methods, are among the factors that influence the regulated levels [42].

In Europe, the maximum levels of mycotoxins are established for the most known and frequently detected ones in section 2 of the Commission Regulation (EC) No. 1881/2006 of 19 December 2006 and its amendments that sets maximum levels for certain contaminants in foodstuffs. Those limits were fixed according to mycotoxins' prevalence and toxicity, and are established for several molecules, such as AFs, OTA, DON, ZEA, and FMs in many food matrices [6]. This regulation was amended in 2010 by the Commission Regulation (EU) No. 165/2010 of 26 February 2010 which established new AFs maximum levels in foodstuffs. Before the milling process, the levels are expected to be slightly higher, due to the greater fraction of mycotoxins in bran, that are removed during this process, lowering the concentrations to an acceptable level [21,43]. The levels established for cereals by the Commission Regulation (EC) No. 1881/2006 of 19 December are described in Table 1.

Table 1. Adapted from Commission Regulation (EC) No. 1881/2006 and its amendments, establishing the maximum permitted levels of mycotoxins in cereals [6].

Mycotoxins	Maize Unprocessed (μg/kg)	Cereals for Direct Human Consumption (μg/kg)	Baby Foods for Infants and Young Children (μg/kg)	Ref.
AFB1	5	2	0.1	[6]
Sum of AFB1, B2, G1 and G2	10	4	-	[6]
OTA	5	3	0.5	[6]
DON	1750	750 *	200	[6]
ZEA	200	200	20	[6]
T-2 and HT-2 toxin	200 (indicative TDI level)	100	15	[44]
Fumonisins	2000	1000 **	200	[6]

TDI – Tolerable Daily Intake. * for bread the value is 500 μg/kg; ** for breakfast cereals the value is 800 μg/kg.

As a result of the protective layer of husk in paddy rice, low levels of Fusarium toxins were detected, and this cereal does not have a specific maximum limit as maize. No maximum levels of Fusarium toxins (ZEA, FUMs, T-2, and HT-2 toxin) are established for rice but rice is predominant in baby foods for infants and young children formulations that have a specific maximum limit [6]. Due to the harmful effects related to the presence of T-2 and HT-2 toxins in feed and foodstuff, the EC came out with a recommendation ("Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products") where are established the tolerable daily intake (TDI) for some food matrices. Rice and rice products are not included in those ma-

trices because these toxins occur at very low levels in this matrix, and so it was excluded from this recommendation since it does not seem to constitute a health concern [44].

The European Regulation concerning the maximum limits of mycotoxins in foods is more restricted than the rest of the world. Outside the European Union (EU), levels of mycotoxins are regulated according to different legally binding documents, or have no limits at all, depending on the type of mycotoxin and foodstuff. All these limits were described in "Worldwide regulations for mycotoxins in food and feed in 2003 by FAO (2004) [45].

China and India, the main rice producers in the world, have established maximum levels, although those are much higher than those of the EU. China sets a maximum of 10 μg/kg to AFB1 (No limit on the sum of AFs in rice) and in baby food is only 0.5 ug/kg. [46]. In India, the limits for AFs are set at 30 μg/kg, which constitutes a matter of concern to the consumers' health [46]. Still, other countries, such as the USA, Canada, and Japan, do not have maximum limits for all mycotoxins mentioned above (Table 1). For example, the USA only has limits for the sum of Afs, 20 ug/kg and for the DON, 1000 μg/kg, as well as Japan, but in Japan, the sum of AFs is 10 μg/kg [47–51]. In Canada they have the maximum limits in the order of nanograms, for example, DON has a maximum limit of 2 ng/kg [52].

One of the greatest limitations in the regulations is associated with the fact that the maximum limits are set according to the mycotoxins' individual toxicity, not taking into account their co-occurrence and potential synergism.

Due to the high susceptibility of maize to contaminations with *Fusarium*-produced mycotoxins (DON, ZEA, fumonisins) the European Regulation specifies maximum levels for feed and food unprocessed maize (Table 1). The rest of the cereals for direct human consumption, especially rice, have been regulated with more restricted levels in particular if used in baby foods for infants and young children. The knowledge of the occurrence of regulated mycotoxins in rice assumes great importance since rice production is mostly for direct human consumption and simultaneously is highly used in the formulations of baby foods for infants and young children to fulfill their 'gluten-free' claims. In addition, a great number of studies have reported rice contamination by several unregulated mycotoxins, so the establishment of maximum limits for more mycotoxins in specific foods seems to be required.

3. Analytical Methodologies to Determine Mycotoxins

Since their first discovery, many methods have been developed for the analysis of mycotoxins in food, despite the frequent analytical challenges. These challenges include difficulties associated with low-level contamination, complex matrices where contamination occurs, evolving complex extraction procedures, the structural diversity of mycotoxins as well as their co-occurrence. In order to face these challenges, many analytical methods have been developed, although they require continuous improvements in order to support mycotoxin legislation and protect human health and the food and feed industry [4].

Mycotoxin determination in food samples is usually associated with common steps, that include sampling, homogenization, sample preparation (extraction generally followed by clean-up), and lastly detection and quantification [4].

3.1. Sampling

Sampling is considered a key step in mycotoxins analysis since it is fundamental to ensure the accuracy of the results and to decide if the whole food batch is compliant or not [4,53].

Mycotoxins are not distributed homogeneously in food; therefore, the implementation of a rigorous sampling protocol is of great importance, to guarantee that the analyzed sample is representative of the entire bulk. Considering consumer safety and producer protection, many sampling plans have been established [53]. These plans are instituted by regulatory entities, such as the FDA and the EC, that came up with the Commission Regulation No. 401/ 2006 where the sampling and analysis methods (such as the number and amount of samples) for the official control of mycotoxins in foodstuffs are described [54,55].

Processed products usually require simpler sampling procedures, since mycotoxins are less heterogeneously distributed in these products than in raw agricultural commodities [15].

3.2. Extraction and Clean-Up Procedures

Extraction is a step required before most detection and quantification analytical methods [37]. This step is of great importance and consists of the separation of the analytes of interest from the food matrix, frequently followed by a clean-up phase to eliminate possible interferences. In the case of solid food samples, such as rice, the first step consists of the extraction of compounds of interest into a liquid phase, followed by a clean-up step in order to enhance the specificity and sensitivity of the detection method [3].

The mycotoxins' chemical properties, the nature of the food matrix, and the final method for detection that will be used are three main factors that should be considered in the selection of the methods for extraction and clean-up [4].

The most frequently used extraction technique consists of the extraction using organic solvents: liquid-liquid extraction (in case of a liquid sample) and solid-liquid extraction (in case of solid samples) [55]. Solid-liquid extraction (SLE) is commonly used for mycotoxins extraction from grains and cereals, such as rice. The solvent selection must rely on the polarity of the mycotoxins of interest and on the type of matrix. Mycotoxins are usually soluble in organic solvents (such as chloroform, acetone, methanol, and acetonitrile), but barely soluble in water. Fumonisins are an exception and present high-water solubility. A mixture of organic solvents with water or acidic solvent is commonly used since water enhances the penetration of the organic solvents in the food matrix and the acidic solvent has the ability to break the strong bonds between the analyte and protein and sugar present on the food matrix [13,56]. This method is associated with high recoveries; however, the use of large amounts of sample and organic solvents, as well as the need to use time-consuming purification processes to minimize interferences during the determination, are significant limitations [57].

Recent studies have been using solvent extraction methods, such as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE). In comparison with SLE, these methods are faster, require smaller volumes of chemical solvents, and are associated with better extraction efficiencies, although they might be costly. Before further clean-up steps, sample filtration and centrifugation are required to eliminate possible interfering particles [56].

The clean-up step plays an important role, allowing the elimination of the substances that may interfere with the detection of mycotoxins, and consequently improving accuracy and precision. Some clean-up methods have been described, including solid phase extraction (SPE), immunoaffinity columns (IAC), solid-phase microextraction (SPME), matrix solid-phase dispersion, and the quick, easy, cheap, effective, reliable, and safe (QuEChERS) method [55].

SPE consists of extracting mycotoxins dissolved in an extract (mobile phase) and passing through solid support (stationary phase), where the mycotoxins are absorbed, and some matrix components are eluted. Usually a washing step, before elution, can eliminate some other interferents that might also be adsorbed in the stationary phase. In the final step, elution of mycotoxins is achieved with an organic solvent for which they have a stronger chemical affinity. The solid phase selection depends on the polarity of mycotoxins and the type of matrix [20,56]. This technique is described as safe, efficient, and reproducible, although it has some limitations, such as the fact that the sample has to be in a liquid phase, the low selectivity due to matrix effects, and the impossibility of using the same solid support for all mycotoxins [20].

Immunoaffinity columns are composed of activated solid phase support, bound to a given antibody. When the sample extract passes through the column, mycotoxins bind selectively to the column antibodies, while interferents and other matrix components are removed by a subsequent washing step. After that, the mycotoxin is eluted with a miscible solvent, such as methanol, removing them from the column [58]. This method has great selectivity, although it also presents some disadvantages, such as the high cost, the column being limited to single use, and its ability to only isolate a given type of mycotoxins, or a group of structurally related mycotoxins. Beyond that, there is also the risk of antibody denaturation, while in contact with some organic solvents, or the possibility of cross-reactivity and establishment of non-specific interactions [17,58]. IAC are available for the extraction of the most common mycotoxins such as AFs, ZEA, OTA, FUMs, and DON, and some columns allow the simultaneous extraction of different classes of mycotoxins [58]. For more complex samples, sometimes it is required the combination of IAC with other extraction methods like SPE [59].

The sample preparation method QuEChERS has been used for extraction and clean-up of different food matrices prior to the detection of mycotoxins. This technique includes two different phases: an extraction step (solvent extraction) followed by a purification one (dispersive-SPE) [60]. The first step is based on solvent extraction, using acetonitrile in the presence of salts such as magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl), in order to remove water from the organic phase and reduce the number of polar interferences, respectively [56]. For the second phase, a primary/secondary amine (PSA), or C18, is frequently used to retain co-extracted compounds such as lipids, sugars, organic acids, or even some pigments. As described in the name itself, this is a fast, simple, and inexpensive method, that uses small amounts of solvent compared with other methods [56].

A compilation of studies that reported mycotoxin's occurrence around the world is presented in Table 2, along with the respective extraction and purification methods. The most frequently used methods for the extraction step in the compiled studies were QuEChERS, immunoaffinity columns, and SPE, but in the last few years, there has been a growing preference for the QuEChERS method.

Table 2. Extraction procedures to determine mycotoxins in rice and rice products and levels of contamination of rice samples.

Type of Sample	Mycotoxins analyzed	Extraction Method	Extraction Conditions	Number of Samples	Sampling Period	Levels of Contamination (μg/kg)	Conclusions of the Study	Ref.
Organic Rice	OTA	Extraction with MSPD	Sample was blended with the solid phase C8 (2.5 g/1.5 g) until achieving a homogeneous mixture. The mixture was eluted through a column (100	9	April 2005–November 2005	Mean: 2.57 ± 3.43 Range: 2.10–7.60	OTA was present in 4 out of the 9 samples.	[61]

Type of Sample	Mycotoxins analyzed	Extraction Method	Extraction Conditions	Number of Samples	Sampling Period	Levels of Contamination (µg/kg)	Conclusions of the Study	Ref.
			mm × 9 mm i.d. glass column with a coarse frit) using MeOH: FA (99:1, *v*/*v*). The eluate was concentrated using a N_2 steam, filtered and then centrifuged.					
Rice	AFs	SPE	Solvent: ACN: H2O: acetic acid (79:20:1 *v*/*v*/*v*). The supernatant was centrifuged, and a purification step was conducted, diluting the final extract with ACN:water:acetic acid (20:79:1). After a second purification by filtration, the final sample was injected into the UHPLC-MS/MS.	40	January–March 2010	0.15–4.42 (10/40 samples)	80% of the cereal samples were contaminated with at least one mycotoxin; 4% of the samples exceeded the EU regulatory levels for AFs and OTA (4 and 5 µg/kg respectively)	[62]
	OTA					0.2–4.34 (6/40 samples)		
	ZEA					1.5–51.1 (5/40 samples)		
	DON					6.15–34.92 (8/40 samples)		
	FB1					12.59–33.25 (3/40 samples)		
	FB2					12.36–31.19 (3/40 samples)		
	T2					5.88–55.35 (3/40 samples)		
	HT-2					48.18 (1 sample)		
Jasmine Rice	AFs	Immunoaffinity columns	Sample extract: MeOH:H20 (60:40 *v*/*v*) and NaCl. The sample was diluted in distilled water and filtered. IAC: The column was buffered with PBS at a flow rate of 5ml/min. The sample was then eluted using MeOH and distilled water, at a flow rate of 2 ml/min, and collected in an amber glass vial.	-	-	Mean: 11.4 of total aflatoxins (in the absence of *Aspergillus*)	1/3 of the analyzed samples exceeded the levels of AFs tolerated in the EU.	[28]
Rice	AFB1	Immunoaffinity column	Sample extract: MeOH:H20 (80:20 *v*/*v*) and NaCl. After filtration, the extract was	67	-	<LOD–91.7	Most of the analyzed samples exceeded the	[63]
	AFB2					<LOD–12.1		
	AFG1					<LOD–78.7		
	AFG2					<LOD–31.0		

Type of Sample	Mycotoxins analyzed	Extraction Method	Extraction Conditions	Number of Samples	Sampling Period	Levels of Contamination (μg/kg)	Conclusions of the Study	Ref.
	AFs		diluted in phosphate buffered saline (PBS), ad filtered again. IAC: The column was buffered with PBS and then the filtered sample was eluted through the column with ACN at a flow rate of 5 ml/min. The column was washed twice with distilled water and air-dried. After that, the eluate was dried and derivatized, and an aliquot was used for the HPLC analysis.			<LOD - 138.6	levels of AFB1 and AFs (2 and 4 μg/kg, respectively) tolerated in cereals in the European Community	
Rice	Total mycotoxins	QuEChERS	Extraction step: Solvent: ACN:HOAc (99:1 *v*/*v*) Salts: mixture of anhydrous $MgSO_4$, NaCl, $(CH_2COONa)_2{\cdot}2H_2O$ and $C_6H_6Na_2O_7{\cdot}1.5H_2O$ (4:1:1:0.5). After being vortexed and centrifuged, the supernatant was collected in a PTFE tube for the purification step, containing anhydrous magnesium sulfate and a C18 sorbent (This process is imperative to reduce the quantity of lipids and eliminate the excess of water, simplifying the evaporation). After centrifugation, the supernatant was evaporated and reconstituted in MeOH:H_2O (70:30 *v*/*v*). After filtration, the extract was collected into a LC vial.	24	2013	ND	The target mycotoxins were not detected in any of the samples.	[4]
Rice	AFB1, AFB2,	d-SPE, QuEChERS	Extraction step: Solvent: water + 10% FA	20	-	ZEA was detected in 2	The contamination	[64]

Type of Sample	Mycotoxins analyzed	Extraction Method	Extraction Conditions	Number of Samples	Sampling Period	Levels of Contamination (μg/kg)	Conclusions of the Study	Ref.
	AFG1, AFG2, OTA, DON, ZEA, FB1, FB2, HT2, T2		in ACN Salts: mixture of anhydrous $MgSO_4$, NaCl, tri-Na and di-Na Purification step (d-SPE) ACN extract + MgSO4 + C18 + Al-N + PSA. After centrifugation, the extract was evaporated to dryness under a N_2 steam, and reconstituted using mobile phase A:B (1:1 *v*/*v*). The samples were then filtered and collected in a vial for injection.			rice samples and AFB1 was detected in 6 rice samples	levels were below the EU limits for typical foods and feeds.	
Rice	AFB1 AFB2 FB1 OTA ZEA	SPE	The samples were extracted with 20 mL ACN/water/glacial acetic acid (79:20:1, *v*/*v*/*v*. Aliquots of 500 μL extracts were transferred into glass vials containing an equal volume of ACN/water/acetic acid (20:79:1, *v*/*v*/*v*).	65	April 2010 - April 2011	<LOQ–30.83 0.6–1.26 54.48–176.58 0.65–11.54 4.95–215.46	All the samples were contaminated with at least one mycotoxin. 3 rice samples exceeded the limit established in EU and Iran for AFB1 (5 μg/kg); ZEA was detected in 19 out of 65 samples in high levels.	[65]
Rice	T-2 toxin HT-2 toxin	SPE using multi - walled carbon nanotubes as sorbents	The samples were macerated using 10 mL of ACN/water (84:16, *v*/*v*) and then ultrasonicated. After centrifugation, the supernatant was collected and dried using nitrogen gas. The residues were reconstituted in ACN/water (20:80, *v*/*v*) and then diluted with water. This solution was passed through the multi-walled carbon nanotubes sorbents. The	10	-	6.13 (1/10 samples) 11.81 (1/10 samples)	EFSA has established a TDI of 100 μg/kg body weight for the total of T-2 and HT-2 toxins	[66]

Type of Sample	Mycotoxins analyzed	Extraction Method	Extraction Conditions	Number of Samples	Sampling Period	Levels of Contamination (μg/kg)	Conclusions of the Study	Ref.
			cartridges were eluted with MeOH containing 1% FA, and the eluate was evaporated using nitrogen gas. The residues were re-dissolved in ACN/water containing ammonium acetate (30:70, *v*/*v*), filtered and collected in a vial for injection.					
White rice	AFB1	SPE, Immunoaffinity columns	AFs: Solvent: ACN:water (90:10 *v*/*v*) After filtration, the supernatant was diluted with deionized water. IAC: the dilute filtrate was eluted at a flow rate of 3–4 drops/s using HPLC grade MeOH and washed with water. After evaporation under a nitrogen stream, a mixture of ACN:water (1:9 *v*/*v*) was added to the vials. OTA: Solvent: ACN:water (90:10 *v*/*v*) After filtration, the sample was mixed in PBS and filtered using a glass microfiber. After filtration, 10 ml of filtrate were mixed with acetic acid and passed through the IAC. IAC: The sample was eluted with MeOH and collected in a vial.	34	August 2012–March 2013	7.70 ± 0.89	25% of the samples of brown rice were above the maximum permitted level at EU for AFB1, and 32% for total AFs. 19% of the samples of rice and rice products were found positive and 14% were found above the EU maximum content for OTA (5 μg/kg)	[67]
	AFs					11.9 ± 1.20		
	OTA					8.50 ± 0.60		
Brown rice	AFB1			28		8.91 ± 1.20		
	AFs					12.4 ± 0.98		
	OTA					7.84 ± 0.90		
Rice flour	AFB1			30		3.51 ± 1.20		
	AFs					5.20 ± 0.82		
	OTA					4.91 ± 1.53		
Sweet puffed Rice balls	AFB1			22		2.90 ± 0.85		
	AFs					4.30 ± 1.25		
	OTA					3.87 ± 0.75		
Rice cookies	AFB1			28		3.18 ± 0.40		
	AFT					5.40 ± 0.92		
	OTA					3.18 ± 0.60		
Rice sweets	AFB1			21		4.10 ± 1.30		
	AFT					5.70 ± 0.80		
	OTA					5.10		
Rice noodles	AFB1			20		3.60 ± 0.85		
	AFT					3.60 ± 0.85		
	OTA					ND		
Rice bread	AFB1			25		2.40 ± 0.43		
	AFT					2.40 ± 0.43		
	OTA					ND		
Brown rice	AFT	QuEChERS	Extraction step: Solvent: water and HOAc in ACN (10% *v*/*v*) Salts: mixture of anhydrous $MgSO_4$, NaCl, $(CH_2COONa)_2{\cdot}2H_2O$ and $C_6H_6Na_2O_7{\cdot}1.5H_2O$ Centrifugation in order to separate the aqueous	14	-	N.D	6 samples were contaminated with one or more mycotoxins. The levels determined were below the maximum	[37]
	OTA					N.D		
	DON					N.D		
	FB1					2.49- 5.41		
	FB2					4.33		

Type of Sample	Mycotoxins analyzed	Extraction Method	Extraction Conditions	Number of Samples	Sampling Period	Levels of Contamination (μg/kg)	Conclusions of the Study	Ref.
			phase from the organic phase and then collection of the supernatant for the Purification step: C18 silica sorbent, anhydrous magnesium sulfate, PSA and silica. After centrifugation, the supernatant was collected into a vial. After evaporating the remaining ACN and reconstituting in water with a 1:1 (*v*/*v*) ratio of 0.1% (*v*/*v*) FA:MeOH, the sample was filtered and collected in the UHPLC-MS/MS vial				limits of EU regulation.	
Infant cereals based on rice	AFB1	SPE	Solvent: ACN:water: FA (80:19.9:0.1 *v*/*v*/*v*) After centrifugation, the supernatant was transferred into an HPLC vial and a [^{13}C] labelled working solution was added.	20	March 2012–June 2012	1/20 (5.9)	1 sample exceeded the EU limit for AFB1.	[68]
	AFB2					4/20 (1.1 - 5.0)		
	AFG1					ND		
	AFG2					ND		
	DON					7/20 (1.4 - 55.0)		
	HT-2 toxin					ND		
	T-2 toxin					3/20 (1.1 - 3.6)		
	FB1					ND		
	FB2					ND		
	OTA					2/20 (1.3–1.4)		
	ZEN					1/20 (9.0)		
Rice wine	OTA	VADLLME (Vortex-assisted dispersive liquid-liquid microextraction)	After centrifugation, the sample pH was adjusted to 4.0–4.3 using 4M NaOH or HCL solutions. Extraction solvent: dichloromethane Dispersive solvent: ACN The mixture was vortexed. After centrifugation, the sediment phase was evaporated to dryness using a nitrogen stream at 50 °C. The residues were reconstituted in a MeOH/water solution	8	2016	0.20 μg/L (1/8 sample)	The contamination levels did not exceed the maximum residue limit set by EU (2 μg/L)	[69]
	AFs					ND		

Type of Sample	Mycotoxins analyzed	Extraction Method	Extraction Conditions	Number of Samples	Sampling Period	Levels of Contamination (μg/kg)	Conclusions of the Study	Ref.
			(50:50, *v*/*v*) and filtrated through a nylon filter membrane.					
Brown rice	AFB1	Immunoaffinity column	Sample extract: MeOH:Water (80:20, *v*/*v*) with NaCl. After filtration, the solution was diluted in phosphate buffered saline (PBS). IAC: The solution was applied to the IAC at a flow rate of 2–3 mL/min. The column was washed with distilled water, and the sample was eluted with MeOH and diluted with milli Q water	187	-	<LOD– 0.069	Less than 14% of the rice samples were contaminated with aflatoxins, but two of the market samples were well above the maximum tolerable limit.	[70]
	AFB2					<LOD		
	AFG1					< LOD		
	AFG2					<LOD		
	AFs					<LOD–0.069		
Red rice	AFB1					<LOD–63.32		
	AFB2					<LOD–8.591		
	AFG1					<LOD		
	AFG2					<LOD		
	AFs					<LOD–70.91		
Rice	AFs	IAC	Sample extract: Sodium chloride and LC grade MeOH 70%. After filtration, the mixture was diluted in PBS and then filtered again. IAC: elution of the sample with 100% LC grade MeOH and LC grade water	100	2017	4,9 (1 sample)	The level is above the legislated levels.	[29]
	DON	Stable isotope dilution assay	Solvent: ACN:water:FA (80:19.9:0.1 *v*/*v*/*v*). After centrifugation, the supernatant was resuspended in a mobile phase composed by 70% of water:MeOH:acetic acid (94:5:1, *v*/*v*/*v*) and 30% of water:MeOH:acetic acid (2:97:1, *v*/*v*/*v*).			ND (0/100 samples)	ZEA levels were higher in 36% of the samples, than the current maximum limit established by Brazilian and European regulation	
	ZEA					15/100 samples (90,56–126,31)		
Rice	AFB1	QuEChERS	Extraction step: Solvent: ACN Salts: mixture of $MgSO_4$ and NaCl. Centrifugation in order to separate the aqueous phase from the organic phase and then collection of the top	47	April 2013	Mean: 3.9 (<LOQ–14)	Most samples were contaminated with more than one mycotoxin (8 different mycotoxins were detected in 2 rice	[2]
	AFG1					Mean: 3.3 (<LOQ–17)		
	AFs					Mean: 5.8 (<LOQ–33)		
	OTA					Mean: 6.3 (<LOQ–15)		
	FB1+FB2					Mean: 6.0		

Type of Sample	Mycotoxins analyzed	Extraction Method	Extraction Conditions	Number of Samples	Sampling Period	Levels of Contamination (μg/kg)	Conclusions of the Study	Ref.
			organic phase for the Purification step: C18 silica sorbent and magnesium sulfate After centrifugation, the supernatant was collected into a vial. After evaporating the remaining ACN and adding MeOH, the sample was filtered and collected in a new vial.			(2.7–13)	samples). Contamination levels higher than the EU limit for AFB1 were found in 42% of rice samples and for Aft in 32% of the same samples. OTA levels were also higher than the regulated from the EU.	
	ZEA					Mean: 6.6 (<LOQ–7.5)		
Ready to eat rice	DON	QuEChERS	Extraction step: Solvent: ACN Salts: mixture of $MgSO_4$ and NaCl. Purification step: Anhydrous $MgSO_4$ and a C18 silica sorbent. After centrifugation, the extract was filtered using a syringe nylon filter, into the LC-MS/MS vial; For GS-MS/MS the supernatant was evaporated to dryness using a nitrogen flow.	38	September 2016 - December 2016	0.29	All levels were in accordance with the EU legislation	[71]
	HT-2 toxin					3.47		
	T-2 toxin					0.52		
	ZEA					0.13		
	AFG2					0.17		
Polished rice	AFB1	QuEChERS	Extraction step: Solvent: ACN aqueous solution (95:5, *v:v*) Salts: anhydrous magnesium sulfate and sodium chloride. Purification step: After vortex and centrifugation, the supernatant was collected and filtered into the LC-MS/MS vial		78	2 samples (0.003-0.14)	The levels of AFB1 were lower than the regulation limit in EU (2 μg/kg)	[12]
Unhusked rice					22	N.D.		
Polished rice	AFB1	QuEChERS	Extraction step: Solvent: ACN aqueous solution (95:5, *v:v*) Salts: anhydrous magnesium sulfate and sodium chloride.	78	-	2 samples (0.003–0.14)	The levels of AFB1 were lower than the regulation limit in EU (2 μg/kg)	[12]
Unhusked rice				22		N.D.		

Type of Sample	Mycotoxins analyzed	Extraction Method	Extraction Conditions	Number of Samples	Sampling Period	Levels of Contamination (μg/kg)	Conclusions of the Study	Ref.
			Purification step: After vortex and centrifugation, the supernatant was collected and filtered into the LC-MS/MS vial					
Rice	AFB1	QuEChERS	Extraction step: Solvent: ACN containing 1% acetic acid Salts: mixture of anhydrous magnesium sulfate and sodium chloride. Purification step: Anhydrous magnesium sulfate and a C18 sorbent. After vortex and centrifugation, the supernatant was collected and filtered into the LC-MS/MS vial	144 (bulk sample > 0.5 kg)	October 2016 - September 2017	13/144 samples (ND–93 μg/kg)	The levels of AFB1 were lower than the regulation limit in Vietname (5 μg/kg), but higher than the EU limits (2 μg/kg)	[27]
	FB1					3/144 samples (ND–675)		
	OTA					ND		
	ZEA					ND		

Legend: ACN—acetonitrile; AFB1—Aflatoxin B1; AFB2—Aflatoxin B2; AFG1—Aflatoxin G1; AFG2—Aflatoxin G2; AFs—Total aflatoxins; C8—octysilica; $(CH_2COONa)_2$ $2H_2O$—sodium citrate tribasic dihydrate; $C_6H_6Na_2O_7 \cdot 1.5H_2O$—sodium citrate dibasic sesquihydrate; DON—Deoxynivalenol; d-SPE—Dispersive Solid Phase Extraction; EFSA—European Food Safety Authority; EU—European Union; FA—Formic Acid; FB1—Fumonisin B1; FB2—Fumonisin B2; GC—Gas Chromatography; HCl—hydrogen chloride; HOAc—Acetic Acid; HPLC—High Performance Liquid Chromatography; IAC—Immunoaffinity Column; LC—Liquid Chromatography; LOD—Limit of Detection; MeOH—methanol; $MgSO_4$—Magnesium Sulfate; MSPD—matrix solid phase dispersion; NaCl—Sodium Chloride; NaOH—Sodium hydroxide; ND—Not Detected; OTA—Ochratoxin A; PBS—phosphate buffered saline; PSA—Primary/Secondary amine; SPE—Solid Phase Extraction; TDI—Tolerable Daily Intake; UHPLC-MS/MS—Ultra High Performance Liquid Chromatography coupled with tandem mass spectrometry; ZEA—Zearalenone.

OTA contamination was found in levels higher than those permitted in cereals, in multiple studies [2,61,67]. Aflatoxin levels were also found to be above the permitted limits, according to some studies [2,27,62,70].

By exploring Table 2, we are once again threatened with the prevalence and unavoidability of mycotoxins' contamination, since more than one study reported the contamination with at least one mycotoxin in over 80% of the analyzed samples [62,65]. Moreover, methods that have shown to be efficient in removing fungal from foodstuffs, might not be efficient in removing mycotoxins, since Ruadrew et al. found that 1/3 of the analyzed samples were contaminated with aflatoxins, in the absence of Aspergillus [28].

The sample with the greatest mycotoxins levels found in this literature review was reported by Suarez-Bonet et al. in a sample of rice from Spain [63]. The maximum levels of AFB1 and total aflatoxins were respectively 91.7 and 138.6 μg/kg, which far exceed the regulated limits, and the fact that those samples were cultivated in temperate climate region (Mediterranean, Spain) enhances the fact that this is a worldwide problem [63]. The highest contamination with OTA was reported by Manizan et al. in a sample of 15 μg/kg [2]. Furthermore, Manizan also emphasized the co-occurrence of mycotoxins, by finding 8 different mycotoxins in two rice samples [2].

3.3. Analytical Methods

3.3.1. Immunochemical Methods

The immunoassay technology has proven to offer many advantages in mycotoxins determination, through the development of simple, efficient, and sensitive methods, based on antibody-antigen reactions. Among these methods are included enzyme-linked immunosorbent assay (ELISA), flow injection immunoassay (FIIA), lateral flow immunoassay (LFIA), flow immunoassay, and chemiluminescence (CL) [72].

CL has already been applied in the determination of mycotoxins in maize samples and consists of the production of fluorescence as a result of a chemical reaction [73]. The most reported advantages are the use of simple instrumentation and the low detection limits obtained [74,75].

ELISA is probably the most frequently used of all published immunological-based methods for mycotoxins determination. ELISA kits are available for the detection and quantification of all major mycotoxins and provide rapid screening results, without the need for clean-up and concentration steps, which makes possible its use in field conditions [58].

This technique is based on the interaction between mycotoxins and antibodies marked with toxin-enzyme conjugate for multiple binding sites. The level of color developed is dependent on the amount of antibody-bound toxin-enzyme conjugates. There are two types of ELISA tests: direct and indirect. Direct ELISA provides quick results and, because it uses only one antibody, it reduces cross-reactivity reactions. However, the direct method is associated with less sensitivity, due to the difficulty of signal amplification on the primary antibody. Indirect ELISA recurs to labeled secondary antibodies, providing higher sensitivity, due to signal amplification [71]. This method is specific, rapid, and easy to use, although it has some disadvantages, including the possibility of cross-reactivity occurrence and dependence on a specific matrix (since matrix effect or interference may induce under or overestimation of mycotoxins) and contamination level [15]. Moreover, each kit is designed for a single use and detects only one mycotoxin. In addition, it can become costly when there is the need to identify various mycotoxins and perform multiple tests. High-performance liquid chromatography (HPLC) analysis is often used as a confirmation method after ELISA e CL [58].

3.3.2. Chromatographic Techniques

Chromatographic methods are the most frequently used for mycotoxins analysis in food samples [53].

Thin layer chromatography (TLC) is commonly used as a rapid screening technique in the analysis of some mycotoxins. Thus, recent investigation has been focusing on the application of methods that allow the detection and quantification of multiple mycotoxins with high selectivity and sensitivity, and the achievement of more accurate results [20].

In order to accomplish that, many other techniques have been developed like HPLC coupled with mass spectrometry (MS), fluorescence (FLD), diode array (DAD), or ultraviolet (UV) detectors. Moreover, gas chromatography (GC) coupled with MS, flame ionization (FID), or electron capture (ECD) detectors have been applied in the identification and quantification of volatile mycotoxins like TC. GC is rarely used in the analysis of mycotoxins with low volatility and high polarity since it requires a prior derivatization step [71,76].

Liquid chromatography (LC) is able to separate thermolabile, non-volatile, and substances with different polarities. Moreover, it can differentiate substances with structural similarities, without the need for derivatization steps, that are required in GC [77]. The solid phases placed inside the analytical column in LC can be classified as normal or reverse phases. LC in the normal phase consists of the elution of mycotoxins through a solid phase (composed of a free or covalent-bounded particle of phenyl, aluminum, or silica

resulting in a polar stationary phase), using a low polarity solvent like acetonitrile. LC methods for aflatoxin determination include both normal and reverse-phase separations, although current methods for aflatoxin analysis typically rely upon reverse-phase HPLC [78]. In the case of RP-HPLC- Fl, a derivatization step is done in order to increase fluorescence intensity. This step can be a precolumn derivatization with trifluoroacetic acid or a postcolumn derivatization with iodine or bromine [68,79]. The reverse phase consists of hydrocarbonated non-polar solid phases (C_8, C_{18}, or short chain of phenyl, cyanopropyl, and n-alkyl bound to silica surface), through which mycotoxins are eluted using binary polar mixtures of water and organic solvents [57]. In Table 3, a summary of liquid chromatography-relevant detection/quantification analytical methods to determine mycotoxins in rice and rice products is presented. HPLC, coupled with an MS detector, was initially applied to the analysis of single mycotoxins, but to date, it is possible to simultaneously quantify many mycotoxins belonging to various chemical families in a single run, which makes it the method of choice for detecting multiple mycotoxins. The simultaneous detection of multiple mycotoxins is particularly desirable because of the co-occurrence of multiple mycotoxins in food. These modern chromatographic methods may also reach sub-ppb levels of the limit of detection when used following suitable preparation and purification steps [68].

Table 3. Liquid chromatography analytical methodologies to determine mycotoxins in rice and rice products.

Mycotoxins Analyzed	Analytical Technique	Conditions	Analytical Column	LOD and LOQ (μg/kg)	Ref.
OTA	LC-FD	Mobile phase: MeOH- FA 0.1M (70:30 *v*/*v*) Flow rate: 0.7 mL/min $\lambda_{Excit\ max}$: 333 nm and $\lambda_{Emis\ max}$: 460 nm	C18 column (150 × 4.6 mm, 5 μm)	LOD: 0.05; LOQ: 0.19	[61]
AFT (AFB1, AFB2, AFG1 and AFG2) OTA ZEA DON FB1 FB2 T2 toxin HT-2 toxin	LC - MS/MS	Mobile phase: A - MeOH; B - water with 0.1% acetic acid; Elution: Gradient; Column temperature: 30 °C; Injection volume: 10–0 μL; Flow: 0.25 mL/min; Electrospray ionization (ESI); Capillary potential: 3 kV; Nebulizing, desolvation and cone gas: nitrogen; Desolvation gas temperature: 400 °C; Source temperature: 120 °C;	C18 column (2.1 × 50 mm, 1.9 μm)	LOD: 0.01 –25; LOQ: 0.02 –40	[62]
Aft (AFB1, AFB2, AFG1 and AFG2)	Fluorescence detector	HPLC-FD Mobile phase: MeOH: Water [40:60 *v*/*v*] adjusted with 350 μl of 4 M nitric acid and 119 mg of potassium bromide per 1 L of mobile phase. Column temperature: 40 °C; Injection volume: 100 μL; Flow: 1 mL/min; $\lambda_{Excit\ max}$ = 362 nm, and $\lambda_{Emis\ max}$ = 426 nm (for AFB1 and AFB2) and $\lambda_{Emis\ max}$ = 256 nm for AFG1 and AFG2)	Inertsil ODS-3V C18 column (4.6 × 150 mm, 5 μm)		[28]

Mycotoxins Analyzed	Analytical Technique	Conditions	Analytical Column	LOD and LOQ (μg/kg)	Ref.
Aft (AFB1, AFB2, AFG1 and AFG2)	Fluorescence detector	HPLC - FD: Mobile phase: Water:ACN:MeOH [65:15:20 *v*/*v*/*v*] degassed for 30 min using vacuum filtration Column temperature: 20 °C; Injection volume: 20 μL; Flow: 1.0 mL/min; $\lambda_{Excit\ max}$ = 360 nm, and $\lambda_{Emis\ max}$ =450 nm	Reverse phase C18 column (4.6 mm × 250 mm, 5 μm)	LOD: 0.4–0.6; LOQ: 1.2 - 1.9	[63]
Total mycotoxins (AF, OTA, T-2 and HT-2 toxins, DON, ZEA, FB1)	LC-ESI-MS/MS	Mobile phase: H2O:MeOH 9:1 with 5 mM ammonium acetate; Elution: Gradient; Column temperature: 30 °C; Injection volume: 20 μL; Flow: 0.3 mL/min; Electrospray ionization (ESI); Ionization mode: Positive; Capillary potential: 2.9 kV; Nebulizing, desolvation and cone gas: nitrogen; Collision gas: argon Cone gas flow: 80 L/h Flow of desolvation gas: 650 L/h; Desolvation gas temperature: 350 °C; Source temperature: 140 °C;	Silica-based reversed-phase C18 Atlantis T3 (150 mm × 2.1 mm × 5 μm)	LOD: 0.11–59.9; LOQ: 0.37 - 199	[4]
AFB1, AFB2, AFG1, AFG2, OTA, DON, ZEA, FB1, FB2, HT2, T2	UHPLC-MS/MS (micromass quattro premier XE triple-quadrupole mass spectrometer)	Mobile phase: A - 0.5% FA in 5mM aqueous ammonium formate; B – ACN:MeOH (1:1, *v*/*v*) Elution: Gradient; Column temperature: 40 °C; Injection volume: 5 μL; Flow: 0.25 mL/min; Electrospray ionization (ESI); Ionization mode: Positive (except for ZEA)	C18 column (1.7 μm, 100 x 2.1 mm), with a pre-column (1.7 μm, 5 × 2.1 mm)	LOD: 0.5–15; LOQ: 1.7 - 50	[64]
AFB1	HPLC - ESI - MS/MS	Column temperature: 25 °C; Nebulizing, desolvation and cone gas: nitrogen; Source temperature: 550 °C	C18 column (5 μm, 30 × 2 mm)	LOD: 0.03 - 2.5; LOQ: 0.3	[65]
AFB2				LOD: 0.03 - 2.5; LOQ: 0.6	
FB1				LOD: 0.03 - 2.5 LOQ: 7	
OTA				LOD: 0.03 - 2.5 LOQ: 0,6	
ZEN				LOD: 0.03 - 2.5 LOQ: 2	
T-2 toxin	UHPLC-MS/MS	Mobile phase: A - Water with 5mmol/L ammonium acetate; B -	C18 column (100 × 3.0 mm,	LOD: 0.01; LOQ: 0.02	[66]

Mycotoxins Analyzed	Analytical Technique	Conditions	Analytical Column	LOD and LOQ (μg/kg)	Ref.
HT-2 toxin		MeOH Elution: Gradient; Column temperature: 40 °C; Injection volume: 5 μL; Flow: 0.4 mL/min; Electrospray ionization (ESI); Ionization mode: Positive; Flow of desolvation gas: 1000 L/h; Flow of cone gas: 30L/h Desolvation gas temperature: 500 °C; Source temperature: 150 °C;	2.7μm)	LOD: 0.03; LOQ: 0.10	
Aflatoxins	HPLC-FD	Mobile phase: ACN:MeOH:water [20:20:60 *v*/*v*/*v*] Flow rate: 1 mL/min $\lambda_{Excit\ max}$: 360 nm and $\lambda_{Emis\ max}$: 440 nm	C18 (4.6 × 250 mm, 5 μm)	AFB1: LOD 0.04; LOQ 0.20; AFB2: LOD 0.10; LOQ 0.30; AFG1: 0.04; LOQ 0.20 AFG2 LOD 0.10; LOQ 0.30	[67]
OTA		Mobile phase: ACN:water:acetic acid [47:51:2 *v*/*v*/*v*] Flow rate: 1mL/min $\lambda_{Excit\ max}$ = 333 nm and $\lambda_{Emis\ max}$ =460 nm		LOD: 0.06; LOQ: 0.18	
Aft (AFB1, AFB2, AFG1, AFG2)	HPLC - ESI - MS/MS	Mobile phase: A - 0.5% (*v*/*v*) FA in water containing 5mM ammonium formate; B - MeOH Elution: Gradient; Column temperature: 40 °C; Injection volume: 10 μL; Flow: 0.3 mL/min; Electrospray ionization (ESI); Ionization mode: Negative and Positive Collision energy: 25eV Cell accelerator voltage: 3V Capillary voltage: 3 kV; Nozzle voltage: 1000V Gas flow: 16 L/min; Gas temperature: 150 °C	C18 column (100 × 2.1 mm, 1.8 μm)	LOD: 0.27 - 0.39; LOQ: 0.82 - 1.2	[37]
OTA				LOD: 0.47; LOQ: 1.5	
DON				LOD: 5.0; LOQ: 15	
FB1, FB2				LOD: 0.48; LOQ: 1.5	
AFB1	HPLC - ESI - MS/MS	Mobile phase: A - 0.1% FA in water; B - 0.1% FA in MeOH, both containing 5mM ammonium formate; Elution: Gradient; Column temperature: 35 °C; Flow: 0.3 mL/min; Electrospray ionization (ESI);	C18 column (100 × 2.1 mm, 1.8 μm)	LOD: 0.1; LOQ: 0.5	[68]
AFB2				LOD: 0.5 LOQ: 1.0	
AFG1				LOD: 0.1; LOQ: 0.5	
AFG2				LOD: 0.5 LOQ: 1.0	

Mycotoxins Analyzed	Analytical Technique	Conditions	Analytical Column	LOD and LOQ (μg/kg)	Ref.
DON	HPLC - MS/MS	Ionization mode: Positive Flow of desolvation gas: 10 L/min; Desolvation gas temperature: 300 °C Nebulizer: 45 psi Sheath gas temperature: 350 °C Flow rate:11L/min Capillary voltage: 3500 V; nozzle voltage: 0 V		LOD:10.0; LOQ: 50.0	
HT-2 toxin				LOD: 1.0; LOQ: 5.0	
T-2 toxin				LOD: 0.05; LOQ: 0.1	
FB1				LOD: 5.0; LOQ: 10.0	
FB2				LOD: 1.0; LOQ: 5.0	
OTA				LOD: 0.1; LOQ: 0.5	
ZEA				N.D.	
AFB1	HPLC - MS/MS	Mobile phase: A - MeOH; B - water with 0.1% FA Elution: Gradient; Column temperature: 40 °C; Injection volume: 5 μL; Flow: 0.3 mL/min; Electrospray ionization (ESI); Ionization mode: Positive Capillary potential: 4.0 kV; Vaporizer temperature: 300 °C Capillary temperature: 350 °C	C18 column (100 × 3.0 mm, 2.7 μm)	LOD: 0.05; LOQ: 0.1	[69]
AFB2				LOD: 0.05; LOQ: 0.1	
AFG1				LOD: 0.1; LOQ: 0.2	
AFG2				LOD: 0.05; LOQ: 0.1	
OTA				LOD: 0.05; LOQ: 0.1	
AFB1	HPLC-FD	Mobile phase: water:ACN:MeOH (6:2:3, *v*/*v*/*v*), containing KBr and nitric acid Elution: Gradient; Injection volume: 20 μL; Flow: 1 mL/min; $\lambda_{Excit\ max}$ = 362 nm and $\lambda_{Emis\ max}$ = 455 nm (for AFG1 and AFG2) and 425 (for AFB1 and AFB2)	C18 column (4.6 × 150 mm, 5 μm)	LOD: 0.016; LOQ: 0.054	[70]
AFB2				LOD: 0.012; LOQ: 0.039	
AFG1				LOD: 0.011; LOQ: 0.038	
AFG2				LOD: 0.004; LOQ: 0.012	
DON	LC-MS/MS	Mobile phase: water:MeOH:ACN (600:200:200, *v*/*v*/*v*) was added to119 mg potassium bromide and 47.6 μL nitric acid Elution: Gradient; Flow: 1 mL/min; Electrospray ionization (ESI); Ionization mode: Positive Capillary temperature: 208 °C; Vaporizer temperature: 338 °C ; Spray voltage: 4500 V; Sheath gas pressure: 60 bar	RP - C18 column (4.6 × 150 mm, 5 μm)	LOD: 0.005; LOQ: 0.025	[29]
ZEA				LOD: 0.01; LOQ: 0.025	
AFB1	UHPLC-MS/MS	Mobile phase: A – 0.1% FA in water; B - MEOH:ACN (1:1 *v*/*v*)	C18 column (1.6μm, 2.1 ×	LOD: 0.05; LOQ: 0.25	[2]

Mycotoxins Analyzed	Analytical Technique	Conditions	Analytical Column	LOD and LOQ (μg/kg)	Ref.
AFG1		Elution: Gradient; Column temperature: 40 °C; Injection volume: 1 μL; Flow: 0.4 mL/min; Electrospray ionization (ESI); Ionization mode: Positive and negative Capillary potential: 1.5 kV; Flow of desolvation gas: 1000 L/h; Desolvation gas temperature: 500 °C; Source temperature: 150 °C;	100 mm)	LOD: 0.12 LOQ: 0.25	
Aft (AFB1, AFB2, AFG1 and AFG2)				-	
OTA				LOD: 0.25; LOQ: 0.62	
FB1 + FB2				LOD: 0.5; LOQ: 1	
ZEA				LOD: 2.5; LOQ: 5	
DON	LC-MS/MS	Mobile phase: A - MeOH (5mM ammonium formate and 0.1% FA); B - water (5mM ammonium formate 0.1% FA; Elution: Gradient; Column temperature: 25 °C; Injection volume: 20 μL; Flow: 0.25 mL/min;	Reverse analytical column C18 (3 μm, 150 × 2 mm ID) and a guard column C18 (4 × 2 mm ID, 3 μm)	LOD: 0.04 - 1.5; LOQ: 0.13 - 5	[80]
HT-2 toxin					
T-2 toxin					
ZEA					
AFG2					
AFB1	LC- MS/MS	Mobile phase: A - aqueous FA solution with ammonium formate; B - ACN Elution: Gradient; Injection volume: 5 μL; Ionization: electrospray ionization (ESI)	ShimadzuShim-pack XR-ODS III column	LOD: 0.03 LOQ: 0.5	[12]
AFB1	LC - MS/MS	Mobile phase: A - MeOH; B- ammonium acetate 10mM Elution: Gradient; ESI mode: positive (for AFB1 and FB1) and negative (for OTA and ZEA) Ionization: electrospray ionization (ESI)	C18 column (4.6 × 150 mm, 2.7 μm)	LOD: 0.1 LOQ: 0.3	[12]
FB1					
OTA					
ZEA					
FB1	RP-HPLC/ESI-TOFMS	Mobile phase: A – water containing 0.1% (*v*/*v*) formic acid; B - MeCN containing 0.1% (*v*/*v*) formic acid Flow: 0.2 mL min^{-1} Column Temperature: 40 °C Elution: Gradient Injection Volume: 1 μL ESI mode: positive ESI parameters: drying gas (N_2) flow and temperature, 10.0 L min^{-1} and 350 °C; nebulizer gas (N_2) pressure, 20 psi; capillary voltage, 3500 V; TOFMS parameters: fragmentor voltage, 170 V; skimmer potential:	ODS H80 (250 mm × 2.1 mm, 4 μm)	-	[81]

Mycotoxins Analyzed	Analytical Technique	Conditions	Analytical Column	LOD and LOQ (μg/kg)	Ref.
		70 V; OCT 1 RF Vpp: 250 V			
FB1	RP-HPLC/ESIITMS	Mobile phase: A – water containing 0.1% (*v*/*v*) formic acid; B - MeCN containing 0.1% (*v*/*v*) formic acid Flow: 0.2 mL min^{-1} Column Temperature: 40 °C Elution: Gradient Injection Volume: 1 μL ESI mode: positive ESI parameters: spray chamber temperature, 55 °C; drying gas (N2) pressure and temperature, 20 psi and 350 °C, respectively; nebulizer gas (N2) pressure, 60 psi; needle voltage, 4000 V; spray shield voltage, 600 V; general parameters: maximum scan times, 2.71; mscans averaged, 3; data rate, 0.37 Hz; multipier offset, 0; Ionization control parameters: target TIC, 100%; maximum ion time, 500,000ms MS2 parameters: capillary voltage, 139 V; RF loading, 75%; isolation window, 3 *m*/*z*; high mass ejection factor, 100%; waveform type, resonant; excitation storage level, 196.4 *m*/*z*; excitation amplitude, 2.83 V; excitation time, 10 ms; RF, modulate; number of frequencies, 1.	ODS H80 (250 mm × 2.1 mm, 4 μm)	-	[81]
AFB1	UHPLC/TOFMS	Mobile phase: A - water/ methanol/acetic acid 94:5:1 (*v*/*v*/*v*); B - methanol/water/acetic acid 97:2:1 (*v*/*v*/*v*) Flow rate: 0.2 mL·min^{-1} ESI mode: positive MS parameters: capillary voltage 6000 V, nebuliser pressure 2 bars, dry gas temperature 200 °C and dry gas flow 7l min^{-1}	C18 column (1.8 μm, 2.1 × 100 mm)	LOD:1 LOQ: 2	[82]
AFB2				LOD: 2 LOQ: 3	
AFB2				LOD: 1 LOQ:1	
AFG2				LOD: 1 LOQ: 3	
OTA				LOD: 9 LOQ: 18	
DON				LOD: 24 LOQ: 48	
FB1				LOD: 16 LOQ: 32	
HT – 2 Toxin				LOD: 20 LOQ: 41	
T-2 Toxin				LOD: 2 LOQ: 5	
ZEA				LOD: 39	

Mycotoxins Analyzed	Analytical Technique	Conditions	Analytical Column	LOD and LOQ (μg/kg)	Ref.
				LOQ: 77	
AFB1	UHPLC/TOFMS	Mobile phase: A - water/ methanol/acetic acid 94:5:1 (*v*/*v*/*v*); B - methanol/water/acetic acid 97:2:1 (*v*/*v*/*v*) Flow rate: 0.2 mL·min^{-1} ESI mode: positive MS parameters: capillary voltage 6000 V, nebuliser pressure 2 bars, dry gas temperature 200 °C and dry gas flow 7l min^{-1}	C18 column (1.8 μm, 2.1 × 100 mm)	LOD: 4 LOQ: 8	[82]
AFB2				LOD: 4 LOQ: 9	
AFG1				LOD: 7 LOQ: 14	
AFG2				LOD: 3 LOQ: 5	
OTA				LOD: 8 LOQ: 17	
DON				LOD: 29 LOQ: 59	
FB1				LOD: 10 LOQ: 19	
HT -2 Toxin				LOD: 7 LOQ: 15	
T-2 Toxin				LOD: 6 LOQ: 11	
ZEA				LOD: 22 LOQ: 45	

Legend: ACN—acetonitrile; AFB1—Aflatoxin B1; AFB2 — Aflatoxin B2; AFG1 — Aflatoxin G1; AFG2 — Aflatoxin G2; AFs — Total aflatoxins; DON —Deoxynivalenol; ESI—Electrospray Ionization; FA —Formic Acid; FB1 — Fumonisin B1; FB2 — Fumonisin B2; FD — Fluorescent Detector; H_2O —Water; HPLC — High Performance Liquid Chromatography; LC — Liquid Chromatography; LOD —Limit of Detection; LOQ —Limit of Quantification; MeOH — Methanol; MS/MS — Tandem mass spectrometry; OTA —Ochratoxin A; RP —Reverse Phase; UHPLC —Ultra High Performance Liquid Chromatography; ZEA —Zea; RP-HPLC/ESI-TOFMS — Reversed-phase High Performance Liquid Chromatography/Electrospray Ionization Time-of-Flight Mass Spectrometry ; RP-HPLC/ESIITMS — Reversed-Phase High Performance Liquid Chromatography/Electrospray Ionization Ion Trap Mass Spectrometry; UHPLC/TOFMS — Ultra High Performance Liquid Chromatography/Time-of-Flight Mass Spectrometry.

Liquid chromatography, mass spectrometry, and fluorescence were the most used techniques. Although HPLC-FLD is preferred for single mycotoxin determination, HPLC-MS/MS is the preferred method for simultaneous determination of multiple mycotoxins, and according to the studies compilation in Table 3, through the years there is a tendency to employ this method.

New technologies are being applied for mycotoxin determination, such as Orbitrap and Time-of-Flight (ToF) detectors. These new technologies allow the obtainment of more accurate results, and specifically, quadrupole-Orbitrap has the ability to confirm the presence of a certain compound by its exact mass and to identify metabolites or compounds that have not yet been monitored [83]. Quadrupole-ToF detectors are also being used in mycotoxin determination since they provide exact mass information and determine the presence of unknown compounds in real samples [84].

The ultra-high performance liquid chromatography/time-of-flight mass spectrometry (UHPLC/TOFMS) method was developed and validated to screen for the presence of mycotoxins in cereal matrices from Ecuador [84]. Paddy rice was contaminated with AFG1, AFB1, DON, FB1, and polished rice was contaminated with AFG1 and HT-2 toxins, as we can see in Table 3. Since no mycotoxin regulations are enforced in Ecuador, the obtained LODs and LOQs were compared with the European maximum permitted limits (Regulation No. 2006/1881/EC) [82].

Fumonisin mycotoxins which are hazardous to humans and animals were produced in a Fusarium verticillioides-infected solid rice culture. To decrease the possibility of the formation of artifacts, the fumonisins were analyzed by reversed-phase high-performance liquid chromatography/electrospray ionization time-of-flight (RP-HPLC/ESI-TOFMS) and ion trap mass spectrometry (RP-HPLC/ESIITMS) immediately after the extraction of the culture material, without any further sample clean-up and, this is essential for the separation, detection, and characterization of unknown, structurally related secondary metabolites such as the mycotoxin isomers. These results can serve as a starting point for more detailed examinations regarding the structure, toxicity, and biosynthesis of FB1 isomers, with a view to providing additional knowledge concerning food and feed safety [81].

The methods used seem to be suitable since both limits of detection (LODs) and limits of quantification (LOQs) are below the maximum limits set by the EU. Moreover, we can observe that through the years, LOD and LOQ levels are becoming lower, which is associated with the evolution of the used techniques, which are becoming more sensitive. By the analysis of Table 3, we can also conclude that the lower LOD and LOQ levels were obtained when using liquid chromatography coupled to triple quadrupole MS, which is the current method of election for mycotoxins' determination in food.

Internal standards are chemical compounds that present a similar behavior to the target substance, and that are not present in the sample, but intend to minimize process losses (like extraction losses). Internal standards are not frequently used in these studies, and only two of the studies did use these standards in their works [13,68]. Because of their chemical and chromatographic similarities to the target toxins, sulfamethoxazole and tagged stable isotopes were chosen as internal standards [13,68].

Regarding the most frequently used detectors in LC, UV detectors have been losing popularity, due to the lack of selectivity and sensitivity, since many interferences absorb in this zone of the spectrum, along with mycotoxins. Diode array detector (DAD), although allows a complete spectrum of all wavelengths, is associated with low sensitivity levels. For mycotoxins that present natural fluorescence (some aflatoxins and OTA), or for those that are fluorescent after derivatization, fluorescence detectors are also a good option since they present high sensitivity and selectivity levels. In spite of those benefits, FLD is being replaced by MS [55].

HPLC coupled with mass-spectrometry has allowed great advances in mycotoxins' analysis since it offers higher sensitivity and selectivity in comparison with other methods, as well as structural information of the analyzed mycotoxin metabolites or degradation products. That is why an increasing number of researchers have been using this technique, not only for identification and quantification but also for toxicokinetic and metabolism studies [85,86]. The mass spectrometer ionizes the molecules and identifies them based on their mass-to-charge ratio (*m*/*z*). Based on the ionization technique, different interfaces have been applied in the detection of mycotoxins, such as atmospheric pressure chemical ionization (APCI), atmospheric pressure photo-ionization (APPI), and electrospray ionization (ESI) [87]. Moreover, there are multiple types of mass analyzers, such as triple quadrupole (QqQ), ToF, and ion trap. Each mass spectrometer presents advantages and disadvantages, and its selection is dependent on the purpose of the analysis. QqQ is mainly used in routine analysis, due to its selectivity, robustness, and repeatability, although it is not able to determine unknown compounds. For that purpose, there are other developed instruments such as ToF detectors (which provide exact mass through high-resolution mass spectrometry) or ion trap detectors (which offer a fragmentation schedule, allowing unambiguous identification of the compound) [17]. Triple quadrupole (ESI) is the most commonly used in mycotoxin analysis. ToF and Orbitrap analyzers are becoming more popular due to their high resolution and high accuracy but ToF is more frequently used [4]. In Table 4 we can see a comparison of MS/MS systems, such as TQ MS (Tripe Quadrupole MS), Q-TOF MS (Quadrupole Time-of-Flight MS), and Orbitrap MS.

Table 4. Comparison of MS/MS systems (TQ MS, Q-TOF MS and Orbitrap MS), adapted from [88,89].

	Strengths	Limitations
TQ MS	Highest sensitivity (MRM) Wide dynamic range of detection Lower cost	Low mass resolution
Q-TOF MS	High mass resolution Wide mass range Medium dynamic range of detection High sensitivity	Low sensitivity than TQ MS MRM mode
Orbitrap MS	High mass resolving power (up to 200,000) Increased space- charge capacity at higher masses due to the independence of trapping potential and larger trapping volume (in contrast to FTICR and quadrupole traps) High mass accuracy (1–2 ppm) High dynamic range (around 5000)	Expensive

Legend: TQ-MS- Triple quadrupole MS; Q-TOF MS- Quadrupole Time-of-Flight MS.

3.4. Biosensors

Since the first article was published in the biosensor area in 1962, great efforts have been made to their commercialization and use in medicine, pharmacy, agriculture, the food industry, and environmental monitoring [90].

In general, biosensors contain biological or biologically derived sensing elements to detect specific bio-analytes integrated with a transducer in order to convert biological signals into electrical signals [91].

Biosensors with point-of-care features are a promising tool for mycotoxins detection, and many researchers focused on developing disposable biosensors [92].

Related to physicochemical properties of mycotoxins (e.g., fluorescence) or the type of transduction, three groups of biosensors are mostly used: electrochemical (potentiometric, amperometric, and impedimetric), optical (surface plasmon resonance (SPR), and fluorescence) and piezoelectric (quartz crystal microbalance (QCM)). Most of the importance of biosensors relies on their high sensitivity and specificity with minimum sample treatment. Electrochemical biosensors are predominant among the above groups [24,29,89,91].

Furthermore, to improve biosensors' sensitivity, a wide variety of metal nanoparticles, carbon nanotubes (CNTs), nanofibers, and quantum dots (QDs) are used due to their simplicity, physiochemical malleability, and high surface areas [87,92].

The electrochemical biosensors are based on potentiometric, amperometric, and impedimetric detection methodologies. The potentiometric sensor requires two (working and reference) or three (working, reference, and counter) electrode systems, and the recognition event is provided by the changes in the circuit potential between working and reference electrodes. The amperometric sensor, similarly to the potentiometric requires a two or three-electrode system [91]. Electrochemical biosensors are a serious alternative to more complex official instrumental techniques such as HPLC coupled to FLD or MS detectors and provide additional benefits allowing reduced costs and shortening analysis time [24,29].

Optical biosensors provide a powerful and attractive alternative to conventional analytical methods such as ELISA and chromatographic techniques which are widely

used for the detection of mycotoxins [90]. Optical biosensors can employ numerous optical methods to detect an analyte of interest [90]. Those methods are usually based on light absorbance, fluorescence, light polarization, and rotation or vibration spectroscopy measurements, such as SPR and fluorescence, approaches like fluorescence resonance energy transfer (FRET) [86,87]. The SPR system utilizes a thin metal (silver or gold) film between two transparent media with different refractive indices, such as glass prism and sample solution. The SPR method detects alterations in the surface layer refractive index in contact with the sensor chip. In the FRET system, the energy is transferred from an excited donor fluorophore to nearby acceptor species. The acceptor and donor in the FRET can be designed in biunique or one-to-multiple manners, ensuring the simultaneous application of multiple mycotoxin detection [91].

The QCM transducer consists of thin gold-plated crystal quartz, where electrodes are placed. Molecular recognition and a binding event in the electrode surface lead to mass alteration and specific vibrations when an electric signal is sent by the quartz, which results in inducing alterations in the resonant frequency [91].

The development of universal biosensing systems and multiplex assays is another trend in the development of mycotoxin biosensors. Although it can be achieved in many cases by replacement of bioreceptor, the number of appropriate multianalyte biosensors is very limited [90]. The results are obtained relatively quickly, as the samples do not need to be shipped and analyzed at laboratories. It also prevents slowing down the food production process. The main limitations of these methods are matrix interference, antibody cross-reactivity, and the necessity of matrices' validation [91].

4. Mycotoxin Contamination in Rice

In the EU, the RASFF allows a quick and simple share of information, between food safety entities and the EC members, about food and feed hazards, such as contamination by mycotoxins, pesticide residues or other contaminants, pathogenic microorganisms, or heavy metals [4]. Every time contamination by mycotoxins or other food hazards is found, the RASFF member state that discovered it releases a market notification [92]. RASFF notifications can be provided by different entities, such as non-official market controls, industrial companies controls, border controls, and consumers, or they might even be reported by countries outside the EU [93].

RASFF is a valuable tool, not only because it allows the identification of emerging food safety risks, but it is also possible to check the most frequent occurrences in a certain period [92].

According to RASFF, mycotoxins are the basis of a great number of notifications, being one of the main cited hazards during the last decade. In 2019, 553 notifications were emitted referring to mycotoxins in foodstuffs, and around 84.6% corresponded to AFs contamination [92,94].

Table 5 summarizes the reported notifications related to mycotoxins contamination in raw rice grain (brown, white) and rice flours since 2019. According to this table, since 2019 over 86 occurrences classified as a serious risk were reported, which means the contamination levels exceed the legislated levels, and so they were removed from the market. The highest AFB1 levels reported in this period were found in a batch imported from Pakistan to the Netherlands, where 44 μg/kg was reported for AFB1 and 49 μg/kg for total AFs. These values far exceed the levels regulated by the EC for these mycotoxins in cereals for direct human consumption (2 μg/kg for AFB1, and 4 μg/kg for AFs) [95].

All these findings emphasize the presence and relevance of mycotoxins in food safety discussion and the need for rigorous control for their mitigation in the rice value chain. Moreover, looking at the results we can conclude that there is a higher incidence of notifications in basmati and organic rice. This raises questions: are more risks of rice mycotoxin contamination associated with their origin or organic production?? Additionally, most of the contamination samples were original from countries outside the EU,

which emphasizes the need for stricter control of food products coming from foreign countries.

Table 5. RASFF notifications due to mycotoxins contamination from 2019 to 06/07/2022.

Date	Country	Origin Country	Product	Mycotoxin	Levels (μg/kg)
22/02/2019	Italy	Pakistan	Basmati rice	AFB1	4.3
22/02/2019	Belgium	Italy	Organic brown rice	OTA	14.1
01/03/2019	Belgium	Pakistan	Basmati rice	AFB1	6.8
01/03/2019	Italy	Pakistan	Basmati rice	AFB1	19.9
				AFs	21.6
22/03/2019	Austria	Germany	Organic brown rice	AFB1	7.1
22/05/2019	France	Italy	Basmati rice	AFB1	4.49
02/08/2019	Germany	Netherlands	Basmati rice	AFB1	3.60
05/09/2019	Poland	Myanmar	Parboiled brown rice	AFB1	4.09
24/10/2019	Portugal	Myanmar	Rice	AFB1	19
28/11/2019	Switzerland	Sri Lanka	Roasted red rice flour	AFB1	15.6
				AFs	19
18/12/2019	Switzerland	Sri Lanka	Roasted red rice flour	AFB1	6.8
				AFs	8.2
27/02/2020	Switzerland	Sri Lanka	Parboiled rice	AFB1	3.4
15/06/2020	Sweden	Cambodia	Organic brown rice	AFB1	20.6
03/07/2020	Greece	Pakistan	Basmati rice	AFB1	5.6
				AFs	5.6
07/07/2020	Greece	Pakistan	Basmati rice	AFB1	6.3
				AFs	6.3
07/07/2020	Greece	Pakistan	Basmati rice	AFB1	6.0
				AFs	6.0
31/07/2020	Poland	Pakistan	Long grain brown rice	AFB1	6.54
				AFs	6.54
21/08/2020	Greece	Pakistan	Basmati rice	AFB1	4.6
				AFs	4.6
21/08/2020	Switzerland	United Kingdom	Basmati rice	OTA	8.3
01/09/2020	Switzerland	Sri Lanka	Red rice	AFB1	8.9
				AFs	11
				OTA	10.3
15/10/2020	Germany	India	Basmati rice	OTA	6.23
20/10/2020	Germany	Pakistan	Organic brown basmati rice	AFB1	14.3
				AFs	15.4
02/12/2020	Netherlands	India	Brown basmati rice	AFB1	24
				AFs	27
05/01/2021	Spain	Pakistan	White rice	AFB1	2.2 - 3.1
21/01/2021	Spain	Pakistan	White rice	AFB1	3
22/01/2021	Greece	Pakistan	Basmati rice	AFB1	3.1
28/01/2021	Netherlands	Pakistan	Organic brown basmati rice	OTA	11.2
04/03/2021	Netherlands	Pakistan	Organic brown basmati rice	AFB1	9.1
17/03/2021	Germany	Netherlands	Basmati rice	OTA	5.26
27/04/2021	Germany	Netherlands	Rice flour	AFB1	5.7 ± 2.5
27/05/2021	Germany	India	Basmati rice	OTA	4.94 ± 0.41
06/08/2021	Netherlands	Pakistan	Brown rice	AFB1	44

Date	Country	Origin Country	Product	Mycotoxin	Levels (μg/kg)
				AFs	49
10/08/2021	Belgium	Pakistan	Broken rice	AFB1	8.6
27/08/2021	Belgium	Pakistan	White broken rice	AFB1	8.6
14/12/2021	Switzerland	Sri Lanka	Rice	AFB1	6.3 ± 1.07
				AFs	6.59 ± 1.32
16/12/2021	Germany	Pakistan	Basmati Rice	AFB1	3.96 ± 1.60
06/01/2022	Belgium	Pakistan	Rice bran	AFB1	4.15
07/02/2022	Netherlands	Pakistan	Basmati Rice	AFB1	13
				AFs	15
14/02/2022	Netherlands	Pakistan	Golden sun basmati rice	AFB1	5
17/02/2022	Netherlands	Pakistan	Rice	AFB1	4.2
17/02/2022	Netherlands	Pakistan	Rice	AFB1	7
22/02/2022	Netherlands	Pakistan	Rice	AFB1	7
22/02/2022	Netherlands	Pakistan	Basmati rice	OTA	12
23/02/2022	Netherlands	India	Basmati rice	AFB1	4.2
23/02/2022	Netherlands	India	Basmati rice	OTA	6.8
				AFB1	3.1
25/02/2022	Netherlands	India	Basmati rice	AFB1	3.2
25/02/2022	Netherlands	India	Basmati rice	AFB1	3.4
28/02/2022	Belgium	Pakistan	Rice	AFB1	5.3
				AFs	6.5
02/03/2022	Netherlands	Pakistan	Rice	AFB1	7.3
10/03/2022	Netherlands	Pakistan	Super basmati brown rice (husked rice)	AFB1	11
				AFs	11
10/03/2022	Netherlands	Pakistan	Rice	AFB1	9.7
				AFs	9.7
11/03/2022	Netherlands	Pakistan	Super basmati brown rice (husked rice)	AFB1	4.7
11/03/2022	Netherlands	Pakistan	Super basmati brown rice (husked rice)	AFB1	14
				AFs	14
14/03/2022	Italy	India	Basmati rice	AFs	4.9 ± 2.0
14/03/2022	Netherlands	Pakistan	Super kernel basmati brown rice	AFB1	5.6
15/03/2022	Italy	Pakistan	Rice	AFB1	4.6 ± 2.0
15/03/2022	Italy	Pakistan	Rice	AFB1	7.2 ± 3.2 *
				AFS	7.9 ± 3.2 *
24/03/2022	Greece	Pakistan	Rice	AFB1	10.7 ± 2.1
				AFs	10.7 ± 2.1
29/03/2022	Netherlands	Pakistan	Rice	AFB1	10
				AFs	10
29/03/2022	Cyprus	India	Basmati rice	AFB1	5.82
31/03/2022	Netherlands	Pakistan	Rice	AFB1	12
				AFs	13
07/04/2022	Netherlands	Pakistan	Rice	AFB1	24
				AFs	26
07/04/2022	Netherlands	Pakistan	Rice	AFB1	15
				AFs	16
07/04/2022	Netherlands	Pakistan	Rice	AFB1	19
				AFs	20

Date	Country	Origin Country	Product	Mycotoxin	Levels (μg/kg)
13/04/2022	Netherlands	Pakistan	Super basmati brown rice (husked rice)	AFB1	18
				AFs	20
15/04/2022	Netherlands	Pakistan	Super kernel basmati brown rice	AFB1	8
15/04/2022	Netherlands	Pakistan	Super basmati brown rice	AFB1	5.1
19/04/2022	Netherlands	Pakistan	Rice	AFB1	11
27/04/2022	Netherlands	Pakistan	Super basmati brown rice	AFB1	9.1
				AFs	9.1
03/05/2022	Netherlands	Pakistan	Super basmati brown rice	AFB1	6.8
03/05/2022	Netherlands	Pakistan	Super kernel basmati brown rice	AFB1	7.2
04/05/2022	Netherlands	Pakistan	Super basmati brown rice	AFB1	8.5
				AFs	8.5
12/05/2022	Netherlands	Pakistan	Basmati brown rice (husked rice)	AFB1	11
				AFs	11
12/05/2022	Netherlands	Pakistan	Super basmati brown rice (husked rice)	AFB1	5.1
12/05/2022	Netherlands	Pakistan	Super basmati brown rice (husked rice)	AFB1	4.7
12/05/2022	Netherlands	Pakistan	Super basmati brown rice (husked rice)	AFB1	48
				AFs	53
12/05/2022	Ireland	India	Basmati rice	OTA	6.3 ± 0.2
18/05/2022	Netherlands	Pakistan	Rice	AFB1	23
				AFs	25
20/05/2022	Netherlands	Pakistan	Husked brown rice	AFB1	8.2
				AFs	8.2
20/05/2022	Cyprus	India	Basmati rice	OTA	16.5
27/05/2022	Netherlands	Pakistan	Super basmati brown rice	AFB1	7.1
01/06/2022	Spain	Pakistan	Basmati rice	AFB1	5.6 ± 24.2%
				AFs	5.6 ± 24.2%
20/06/2022	Slovenia	Pakistan	Basmati brown rice	AFB1	13.2 ± 2
				AFs	14 ± 2
30/06/2022	Netherlands	Pakistan	Rice	AFB1	7.1
01/07/2022	Netherlands	Pakistan	Rice	AFB1	4.7
04/07/2022	Netherlands	India	Rice	OTA	6.4
06/07/2022	Netherlands	India	Rice	OTA	9.2

Legend: Notifications of mycotoxins contamination in rice and rice products from 2019 to 2021; Adapted from RASFF portal. * mg/kg.

5. Contamination Mitigation

Since mycotoxin-producing fungi may affect rice in multiple stages, many strategies to overcome this problem have been developed, from prevention of their occurrence to decontamination methods [96].

One of the developed strategies to reduce mycotoxigenic fungi in the field is chemical control. Although chemicals have shown to be successful in crop protection, they are associated with undesirable effects. By acidifying the soil, they may interfere with the plant's growth, as they decrease the occurrence of beneficial organisms. Furthermore, nowadays there is an increasing pressure to reduce the use of insecticides, fungicides, and herbicides, in order to achieve higher agricultural sustainability levels [21].

Postharvest strategies are associated with the application of proper storage conditions because almost all mycotoxin contamination in rice grain is associated with inadequate storage. Therefore, the application of suitable packaging practices (such as the use

of ultra-hermetic airtight containers), temperature and humidity control, and ventilation efficiency are essential to avoid fungal growth and mycotoxins accumulation [90]. However, brown rice has more nutritional value which motivates the search for other detoxification strategies.

The distribution and concentration of mycotoxins, as well as their physical and chemical properties, suffer modifications during processing, which may lead to a variation in their toxicity levels [17]. Therefore, it is of great importance to understand the impact and phases where those variations occur. Some studies have found higher levels of AFB1 and AFB2 in brown rice and bran, and lower levels in white rice, suggesting the most relevant step to overcome this mycotoxin is bran removal [95].

Since in some cases mycotoxin occurrence cannot be avoided, some decontamination methods have been developed. These methods must be safe, environmentally friendly, effective, and have a good cost-benefit relationship. A decontamination strategy, to be considered effective, must be able to inactivate, remove or destroy the mycotoxins, and retain the nutritional properties of the foodstuff. Moreover, it must not alter the product's technological properties, or form other toxic substances or metabolites [95]..

In the case of aflatoxins, several detoxification strategies have been proposed, such as physical methods of separation, thermal inactivation, irradiation, adsorption from solution, solvent extraction, microbial inactivation, and fermentation, as well as chemical detoxification methods [97].

In summary, three types of decontamination methods may be applied: physical, chemical, or biological. However, there is no single technique that has proved effective against the wide array of mycotoxins that might occur simultaneously in a food commodity. The methods should be able to completely destroy, inactivate, or remove the toxin along with any residual fungal spores. At the same time, it must preserve the nutritional value and the technological properties of the commodity. In short, in Table 6 we have the advantages and disadvantages of the different methods [95,97,98]].

Table 6. Different decontamination means of mycotoxins in food, their advantages, and disadvantages, adapted from [98].

	Physical Decontamination	**Chemical Decontamination**	**Biological Decontamination**
Examples	Sorting Sieve cleaning Density segregation Washing De-hulling Steeping Extrusion cooking Steam heating Infrared heating Microwave heating Radio frequency heating Irradiation Cold plasma Photocatalytic detoxification	Organic acids Hydrochloric acid Ammonium hydroxide Hydrogen peroxide Sodium bisulphite Chlorinating agents Ozone Formaldehyde Natural substances such as herbs, spices, and their extracts	Bacteria Yeasts Mold Algae
Advantages	Effective against some mycotoxins Low change in food properties	Effective against some mycotoxins Affordable	Effective against some mycotoxins Inexpensive Environment friendly

	Does not involve usage of chemicals		Does not involve usage of chemicals
Disadvantages	Impractical Might be limited to large-scale industries with sophisticated equipment Time-consuming Expensive In case of thermal treatment possible changes in color and food quality	Possible health effects Formation of toxic byproducts Enhancing bioavailability of masked mycotoxins Time consuming Environmentally toxic	Time consuming Impractical More effective in controlled laboratory settings

Physical methods comprise the separation of damaged or contaminated crops from healthy ones and they include methods such as sorting, sieve cleaning, density segregation, washing, dehulling, and steeping that help reduce the concentration of mycotoxins. They also include the destruction of mycotoxins through heat treatment and irradiation. The study of Reduction in Aflatoxin Content of Feed and Food [99] shows that the removal method of external grain parts (dehulling, polishing) was effective in reducing 88–92% of aflatoxins, high moisture thermal treatment (roasting, extrusion, cooking, high-pressure cooking, instant catapult steam explosion) was effective in reducing 25–88% of aflatoxins in rice and UV-light, near-infrared radiation reduced <99 % of aflatoxins in rice. Although physical techniques seem acceptable since there would be limited change afterward in the properties of the rice grains, their usage is still considered unpractical and limited only to large-scale industries since they might be time-consuming and expensive [95,98].

Other alternatives are chemical methods that employ chemical compound treatments with acids, alkalis, and reducing and oxidizing agents, that are either of organic or synthetic nature. Chemical treatment has shown to be effective in the removal of some mycotoxins, however, some chemicals may not show enough effectiveness in the removal of high levels of mycotoxins. These methods include the use of chlorination agents, oxidants, or hydrolytic agents, and also the use of biological agents such as plant extracts and essential oils (EOs) [100].

Treatment with ozone was shown to be promising since it can degrade mycotoxins through reacting with bonds in the mycotoxin chemical structure especially double bonds in mycotoxins such in AFB1 [98].

Although quite a few synthetic preservatives have been identified, their continuous use has been associated with some disadvantages, such as health and environmental issues, an increase in fungal resistance, and allergic reactions. Therefore, the tendency to use natural compounds, such as EOs, to preserve foodstuffs has been increasing in the last decades and is gaining cumulative interest because of their traditional use in pharmaceutics [98,99]. EOs have shown to exhibit biological antifungal, antibacterial, and antioxidant properties, and have already been applied in a wide range of industries, including the pharmaceutical, agricultural, and food ones [101]. Some studies have been performed in order to establish EOs effects on mycotoxigenic fungi and mycotoxin synthesis, and the results indicated that thyme and oregano EOs have been commonly used against fungi producers of aflatoxins, *A. flavus* and *A. Parasiticus* [101,102]. Moreover, cinnamon and cinnamaldehyde have been revealed to present antifungal activity against *Aspergillu*s and *Fusariu*m genera, and significant antimycotoxigenic activity against DON, AFB1, ZEA, and OTA. Great results using oregano extracts have also been reported against OTA [103–105]. Regardless of all these advantages, EOs also present some issues, such as the occurrence of undesirable organoleptic effects and their low potency. In

an attempt to overcome their undesirable organoleptic effects, research studies have developed new approaches such as encapsulation and coating. Their low potency is being overcome through their association with other antimicrobial compounds, to obtain synergistic effects [106].

Both physical and chemical methods present disadvantages, since complete decontamination is not achieved, and these methods are associated with high costs and nutritional loss [102].

Lastly, another strategy developed to reduce mycotoxigenic fungi contamination, comprises the use of microorganisms. This biocontrol method is based on multiple mechanisms, including their ability to compete with pathogens for space and nutrients, produce antimicrobial compounds, induce host resistance to the disease, or directly antagonize the pathogen. Lactic acid bacteria have been used as biocontrol agents since they seem to have a great potential to control fungal diseases. A couple of strains of *Streptomyces corchorusii* and *Burkholderia gladioli* have also been studied because of their abilities to produce cell wall degrading enzymes and to inhibit *A. flavus* growth, respectively [21].

Some of these methods have already been applied to rice in order to mitigate mycotoxin contamination, through the application of field and postharvest good practices. Rice processing also constitutes an important step and seems to reduce mycotoxin content, although it cannot fully eliminate these contaminants [21]. EOs have also already been applied in rice, in order to manage mycotoxin formation and fungal growth, and seem to constitute an effective technique. One of the studies was performed by Wan et al. (2019), in order to evaluate the effects of thyme, lemongrass, cinnamon, peppermint, and clove EOs in the production of DON in contaminated rice. These samples were incubated for 5 days in the presence of the previously referred EOs and, by the end of that period, the results indicated several reductions in mycotoxin production [102].

Another study reports chemically characterized Myristica fragrans essential oil (MFEO) as a plant-based food preservative against fungal and aflatoxin B1 (AFB1) contamination of scented rice varieties. Myristica fragrans Houtt. (family: Myristicaceae) is an aromatic plant indigenous to Indonesia, and also cultivated on large scale in India. In this paper, the authors see the efficacy of MFEO against isolated fungal species and AFB1 secretion by AF LHP R14 cells, the antioxidant activity of MFEO, and the phytotoxicity assay of MFEO. Additionally, it shows that due to the pronounced antifungal, antiaflatoxigenic, antioxidant activity, and nonphytotoxic nature, the MFEO can be recommended as a plant-based food preservative for the protection of scented rice varieties and other agri-food commodities from fungal and mycotoxin contamination as well as oxidative biodeterioration [107].

In addition, another paper shows that *Apium graveolens* essential oil (AGEO) and their major components linalyl acetate(LA) and geranyl acetate GA (1:1:1) can inhibit the growth of a wide range of toxigenic food-borne molds as well as AFB1 secretion and recommends its possible deployment for development of novel plant-based safe food preservative [108].

6. Conclusions and Future Perspectives

Mycotoxins and their fungal producers constitute a great public health issue, with AFB1 being in the spotlight of those concerns since it was considered by IARC as a group 1 carcinogen. Since the prediction of mycotoxins contamination is very dependent on climate change, the key to minimizing their occurrence must be based on prevention and control. To do so, the implementation of good agricultural and production practices, along with the adoption of proper process, transport, and storage conditions with control analysis of critical points is fundamental. Although agricultural practices and control methods are in constant evolution, a large number of RASFF notifications are still reported every year due to mycotoxins contamination, with some of the values being far above the legislated levels.

Since 2019, the reported notifications of mycotoxins contamination in rice (86occurrences) and other published results highlight the aflatoxins and OTA levels as a serious risk and a main concern for the rice chain sustainability.

To minimize the exposure to mycotoxins, more sensitive and accurate analytical methods for their determination have been developed. IAC and QuECHERS are the preferred methods for extraction and purification and HPLC-MS/MS is the preferred method for quantification purposes. Considering the continuous evolution of methods, it is expected that these techniques will be replaced by high-resolution mass spectrometers such as Orbitrap and ToF. These detectors are still very expensive, but there is a possibility that in the future they will be less expensive and become progressively more ubiquitous in routine laboratories. The development of screening methods with greater precision and sensibility able to be employed in the field is also expected.

Further investigation is still required in this field in order to better understand the effects of mycotoxin co-occurrence and its potential synergism. Moreover, climate changes have been found to be problematic in this research area, since higher temperature and humidity levels are favorable conditions for fungal growth and mycotoxin production. Therefore, it would be of great importance to carry out more studies in order to evaluate the impact of climate change on rice contamination by mycotoxins.

The legislation itself also requires updating since it establishes the maximum levels for mycotoxins in cereals for direct human consumption, but emergent and masked mycotoxins are not considered.

Rice is one of the most consumed cereals worldwide not only for direct consumption but also for processing into baby foods, resulting in a large exposure to their potential contaminants, consequently, the continuous control of rice mycotoxins occurrence is relevant for their mitigation and avoiding the associated risk to human health.

Author Contributions: Conceptualization, A.S.S. and C.B.; methodology, A.R.S.; software, A.R.S. and F.C.; validation, A.S.S., F.R., A.F., S.B., and C.B.; formal analysis, A.R.S.; investigation, A.R.S.; resources, A.R.S. and F.C.; data curation, A.R.S. and F.C.; writing—original draft preparation, A.R.S.; writing—review and editing, A.S.S., F.R., A.F., S.B., and C.B.; visualization, A.S.S., F.R., A.F., S.B., and C.B.; supervision, A.S.S., F.R., and C.B.; project administration, A.S.S. and C.B.; funding acquisition, C.B. All authors have read and agreed to the published version of the manuscript.

Funding: The study was funded by project TRACE-RICE—Tracing rice and valorizing side streams along Mediterranean blockchain, grant No. 1934, of the PRIMA Programme, supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation. This research was also funded by PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the grant UIDB/00211/2020. F. Carreiró is grateful for her fellowship in the frame of TRACE-RICE project.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Khodaei, D.; Javanmardi, F.; Khaneghah, A.M. The global overview of the occurrence of mycotoxins in cereals: A three-year survey. *Curr. Opin. Food Sci.* **2021**, *39*, 36–42. https://doi.org/10.1016/j.cofs.2020.12.012.
2. Manizan, A.L.; Oplatowska-Stachowiak, M.; Piro-Metayer, I.; Campbell, K.; Koffi-Nevry, R.; Elliott, C.; Akaki, D.; Montet, D.; Brabet, C. Multi-mycotoxin determination in rice, maize and peanut products most consumed in Côte d'Ivoire by UHPLC-MS/MS. *Food Control* **2018**, *87*, 22–30. https://doi.org/10.1016/j.foodcont.2017.11.032.
3. Krska, R. Performance of modern sample preparation techniques in the analysis of Fusarium mycotoxins in cereals. *J. Chromatogr. A* **1998**, *815*, 49–57. https://doi.org/10.1016/s0021-9673(98)00003-x.
4. Pereira, V.; Fernandes, J.; Cunha, S. Mycotoxins in cereals and related foodstuffs: A review on occurrence and recent methods of analysis. *Trends Food Sci. Technol.* **2014**, *36*, 96–136. https://doi.org/10.1016/j.tifs.2014.01.005.
5. Fernandes, P.J.; Barros, N.; Santo, J.L.; Câmara, J.S. High-Throughput Analytical Strategy Based on Modified QuEChERS Extraction and Dispersive Solid-Phase Extraction Clean-up Followed by Liquid Chromatography-Triple-Quadrupole Tandem Mass Spectrometry for Quantification of Multiclass Mycotoxins in Cereals. *Food Anal. Methods* **2015**, *8*, 841–856. https://doi.org/10.1007/s12161-014-9947-y.
6. European Commission. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union* **2006**, *L364*, 5–24. Available online: http://www.efsa.europa.eu/etc/medialib/efsa/ (accessed on 21 July 2022).
7. Ali, N. Aflatoxins in rice: Worldwide occurrence and public health perspectives. *Toxicol. Rep.* **2019**, *6*, 1188–1197. https://doi.org/10.1016/j.toxrep.2019.11.007.
8. Liu, Y.; Wu, F. Global Burden of Aflatoxin-Induced Hepatocellular Carcinoma: A Risk Assessment. *Environ. Health Perspect.* **2010**, *118*, 818–824. https://doi.org/10.1289/ehp.0901388.
9. Mitchell, N.J.; Bowers, E.; Hurburgh, C.; Wu, F. Potential economic losses to the US corn industry from aflatoxin contamination. *Food Addit. Contam. Part A* **2016**, *33*, 540–550. https://doi.org/10.1080/19440049.2016.1138545.
10. Mahdjoubi, C.K.; Arroyo-Manzanares, N.; Hamini-Kadar, N.; García-Campaña, A.M.; Mebrouk, K.; Gámiz-Gracia, L. Multi-Mycotoxin Occurrence and Exposure Assessment Approach in Foodstuffs from Algeria. *Toxins* **2020**, *12*, 194. https://doi.org/10.3390/toxins12030194.
11. Bennett, J.W.; Klich, M. Mycotoxins. *Clin. Microbiol. Rev.* **2003**, *16*, 497–516. https://doi.org/10.1128/CMR.16.3.497.
12. Zhao, Y.; Wang, Q.; Huang, J.; Chen, Z.; Liu, S.; Wang, X.; Wang, F. Mycotoxin contamination and presence of mycobiota in rice sold for human consumption in China. *Food Control* **2019**, *98*, 19–23. https://doi.org/10.1016/j.foodcont.2018.11.014.
13. Azam, S.; Ahmed, S.; Islam, M.N.; Maitra, P.; Islam, M.M.; Yu, D. Critical Assessment of Mycotoxins in Beverages and Their Control Measures. *Toxins* **2021**, *13*, 323. https://doi.org/10.3390/toxins13050323.
14. Marin, S.; Ramos, A.J.; Cano-Sancho, G.; Sanchis, V. Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food Chem. Toxicol.* **2013**, *60*, 218–237. https://doi.org/10.1016/j.fct.2013.07.047.
15. Lee, H.J.; Ryu, D. Advances in Mycotoxin Research: Public Health Perspectives. *J. Food Sci.* **2015**, *80*, T2970–T2983. https://doi.org/10.1111/1750-3841.13156.
16. Rossi, F.; Gallo, A.; Bertuzzi, T. Emerging mycotoxins in the food chain. *Mediterr. J. Nutr. Metab.* **2020**, *13*, 7–27. https://doi.org/10.3233/MNM-190345.
17. Duarte, S.C.; Pena, A.L.S.; de Matos Lino, C. *Mycotoxins & Their Implications in Food Safety*; Future Science Ltd.: Unitec House, 2 Albert Place, London N3 1QB, UK 2014. https://10.4155/9781909453227
18. Liuzzi, V.C.; Mirabelli, V.; Cimmarusti, M.T.; Haidukowski, M.; Leslie, J.F.; Logrieco, A.F.; Caliandro, R.; Fanelli, F.; Mulè, G. Enniatin and Beauvericin Biosynthesis in Fusarium Species: Production Profiles and Structural Determinant Prediction. *Toxins* **2017**, *9*, 45. https://doi.org/10.3390/toxins9020045.
19. Berthiller, F.; Crews, C.; Dall'Asta, C.; De Saeger, S.; Haesaert, G.; Karlovsky, P.; Oswald, I.P.; Seefelder, W.; Speijers, G.; Stroka, J. Masked mycotoxins: A review. *Mol. Nutr. Food Res.* **2013**, *57*, 165–186. https://doi.org/10.1002/mnfr.201100764.
20. Zhang, K.; Banerjee, K. A Review: Sample Preparation and Chromatographic Technologies for Detection of Aflatoxins in Foods. *Toxins* **2020**, *12*, 539. https://doi.org/10.3390/toxins12090539.
21. Gonçalves, A.; Gkrillas, J.L.A.; Dorne, C.J.L.; Dall'Asta, R.C.; Palumbo, R.; Lima, N.R.; Battilani, P.; Venâncio, A.; Giorni, V.P. Pre- and Postharvest Strategies to Minimize Mycotoxin Contamination in the Rice Food Chain. *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 441–454. https://doi.org/10.1111/1541-4337.12420.
22. Prandini, A.; Sigolo, S.; Filippi, L.; Battilani, P.; Piva, G. Review of predictive models for Fusarium head blight and related mycotoxin contamination in wheat. *Food Chem. Toxicol.* **2009**, *47*, 927–931. https://doi.org/10.1016/j.fct.2008.06.010.
23. Leggieri, M.C.; Mazzoni, M.; Battilani, P. Machine Learning for Predicting Mycotoxin Occurrence in Maize. *Front. Microbiol.* **2021**, *12*, 661132. https://doi.org/10.3389/fmicb.2021.661132.
24. Garcia, D.; Ramos, A.J.; Sanchis, V.; Marín, S. Predicting mycotoxins in foods: A review. *Food Microbiol.* **2009**, *26*, 757–769. https://doi.org/10.1016/j.fm.2009.05.014.

25. Marín, S.; Freire, L.; Femenias, A.; Sant'Ana, A.S. Use of predictive modelling as tool for prevention of fungal spoilage at different points of the food chain. *Curr. Opin. Food Sci.* **2021**, *41*, 1–7. https://doi.org/10.1016/j.cofs.2021.02.006.
26. Venkatesh, N.; Keller, N.P. Mycotoxins in Conversation With Bacteria and Fungi. *Front. Microbiol.* **2019**, *10*, 403. https://doi.org/10.3389/fmicb.2019.00403.
27. Do, T.H.; Tran, S.; Le, C.D.; Nguyen, H.-B.T.; Le, P.-T.T.; Le, T.D.; Thai-Nguyen, H.-T. Dietary exposure and health risk characterization of aflatoxin B1, ochratoxin A, fumonisin B1, and zearalenone in food from different provinces in Northern Vietnam. *Food Control* **2020**, *112*, 107108. https://doi.org/10.1016/j.foodcont.2020.107108.
28. Ruadrew, S.; Craft, J.; Aidoo, K. Occurrence of toxigenic Aspergillus spp. and aflatoxins in selected food commodities of Asian origin sourced in the West of Scotland. *Food Chem. Toxicol.* **2013**, *55*, 653–658. https://doi.org/10.1016/j.fct.2013.02.001.
29. Savi, G.D.; Piacentini, K.C.; Rocha, L.O.; Carnielli-Queiroz, L.; Furtado, B.G.; Scussel, R.; Zanoni, E.T.; Machado-De-Ávila, R.A.; Corrêa, B.; Angioletto, E. Incidence of toxigenic fungi and zearalenone in rice grains from Brazil. *Int. J. Food Microbiol.* **2018**, *270*, 5–13. https://doi.org/10.1016/j.ijfoodmicro.2018.02.004.
30. Al-Zoreky, N.S.; Saleh, F.A. Limited survey on aflatoxin contamination in rice. *Saudi J. Biol. Sci.* **2019**, *26*, 225–231. https://doi.org/10.1016/j.sjbs.2017.05.010.
31. Fraga, H.; Guimarães, N.; Santos, J.A. Future Changes in Rice Bioclimatic Growing Conditions in Portugal. *Agronomy* **2019**, *9*, 674. https://doi.org/10.3390/agronomy9110674.
32. IPCC. *Climate Change 2013—The Physical Science Basis; Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Cambridge University Press: Cambridge, UK, 2014. https://doi.org/10.1017/CBO9781107415324.
33. Ostry, V.; Malir, F.; Toman, J.; Grosse, Y. Mycotoxins as human carcinogens—the IARC Monographs classification. *Mycotoxin Res.* **2017**, *33*, 65–73. https://doi.org/10.1007/s12550-016-0265-7.
34. IARC. IARC monographs on the evaluation of carcinogenic risks to humans. *IARC Monogr. Eval. Carcinog Risks Hum.* **2010**, *93*, 9–38. https://doi.org/10.1136/jcp.48.7.691-a.
35. Rawla, P.; Sunkara, T.; Muralidharan, P.; Raj, J.P. Update in global trends and aetiology of hepatocellular carcinoma. *Contemp. Oncol.* **2018**, *22*, 141–150. https://doi.org/10.5114/wo.2018.78941.
36. Mantle, P. Risk assessment and the importance of ochratoxins. *Int. Biodeterior. Biodegradation* **2002**, *50*, 143–146. https://doi.org/10.1016/s0964-8305(02)00079-3.
37. Jettanajit, A.; Nhujak, T. Determination of Mycotoxins in Brown Rice Using QuEChERS Sample Preparation and UHPLC–MS-MS. *J. Chromatogr. Sci.* **2016**, *54*, 720–729. https://doi.org/10.1093/chromsci/bmv244.
38. Soriano, J.; González, L.; Catalá, A. Mechanism of action of sphingolipids and their metabolites in the toxicity of fumonisin B1. *Prog. Lipid Res.* **2005**, *44*, 345–356. https://doi.org/10.1016/j.plipres.2005.09.001.
39. Hussein, H.S.; Brasel, J.M. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* **2001**, *167*, 101–134. https://doi.org/10.1016/s0300-483x(01)00471-1.
40. Zain, M.E. Impact of mycotoxins on humans and animals. *J. Saudi Chem. Soc.* **2011**, *15*, 129–144. https://doi.org/10.1016/j.jscs.2010.06.006.
41. Sobrova, P.; Adam, V.; Vasatkova, A.; Beklova, M.; Zeman, L.; Kizek, R. Deoxynivalenol and its toxicity. *Interdiscip. Toxicol.* **2010**, *3*, 94–99. https://doi.org/10.2478/v10102-010-0019-x.
42. Zmudzki, J.; Wiśniewska-Dmytrow, H. Limits and regulations for mycotoxins in food and feed. *Pol. J. Veter. Sci.* **2004**, *7*, 211–216.
43. European Commission. Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Off. J. Eur. Union* **2010**, *L50*, 8–12. Available online: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:050:0008:0012:EN:PDF (accessed on 21 July 2022).
44. European Commission. Commission Recommedation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products. *Off. J. Eur. Union* **2013**, *L91*, 12–15. Available: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:091:0012:0015:EN:PDF (accessed on 21 July 2022).
45. FAO. Worldwide Regulations for mycotoxins in food and feed 2003. *Food Nutr. Pap.* **2022**, *81*. Available online: https://www.fooddiagnostics.dk/seekings/uploads/Worldwide_mycotoxin_regulations_in_food_and_feed_2003.pdf (accessed on 20 July 2022).
46. Ward, M.; Clever, J. China Releases Standard for Maximum Levels of Mycotoxins in Foods. 2018. https://www.fas.usda.gov/data/china-china-releases-standard-maximum-levels-mycotoxins-foods (acessed on 13 September 2022).
47. Maximum Permitted Levels of Mycotoxins in Food: EU vs. USA|Download Table. Available online: https://www.researchgate.net/figure/Maximum-permitted-levels-of-mycotoxins-in-food-EU-vs-USA_tbl1_324479616 (accessed 12 August 2022).
48. FDA. Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed. Available online: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-action-levels-poisonous-or-deleterious-substances-human-food-and-animal-feed (accessed 12 August 2022).
49. FDA. Guidance for Industry and FDA: Advisory Levels for Deoxynivalenol (DON) in Finished Wheat Products for Human Consumption and Grains and Grain By-Products Used for Animal Feed. Available online: https://www.fda.gov/regulatory-

information/search-fda-guidance-documents/guidance-industry-and-fda-advisory-levels-deoxynivalenol-don-finished-wheat-products-human (accessed 12 August 2022).
50. FDA. Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds. Available online: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-fumonisin-levels-human-foods-and-animal-feeds (accessed 12 August 2022).
51. Romer Labs. Mycotoxin Regulations for Food and Feed in Japan. September 2016. p. 2016. Available online: https://www.romerlabs.com/en/knowledge-center/knowledge-library/articles/news/worldwide-mycotoxin-regulations/ (acessed 12 August 2022).
52. Government of Canada Health Canada's Maximum Levels for Various Chemical Contaminants in Foods. Available online: https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/chemical-contaminants/maximum-levels-chemical-contaminants-foods.html (accessed on 10 May 2021).
53. Shephard, G.S. Current Status of Mycotoxin Analysis: A Critical Review. *J. AOAC Int.* **2016**, *99*, 842–848. https://doi.org/10.5740/jaoacint.16-0111.
54. European Commission. Commission Regulation (EC) No 401/2006 of 23 February 2006 Laying down the Methods of Sampling and Analysis for the Official Control of the Levels of Mycotoxins in Foodstuffs. *Off. J. Eur. Union* **2006**, *70*, 12–34. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R0401&from=en (accessed on 21 July 2022).
55. Cigić, I.K.; Prosen, H. An Overview of Conventional and Emerging Analytical Methods for the Determination of Mycotoxins. *Int. J. Mol. Sci.* **2009**, *10*, 62–115. https://doi.org/10.3390/ijms10010062.
56. Alshannaq, A.; Yu, J.-H. Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food. *Int. J. Environ. Res. Public Health* **2017**, *14*, 632. https://doi.org/10.3390/ijerph14060632.
57. Castillo, J.M.S. Micotoxinas-Alimentos-pdf. Available online: https://www.yumpu.com/en/document/read/64817491/micotoxinas-alimentos-pdf (accessed 20 July 2022).
58. Singh, J.; Mehta, A. Rapid and sensitive detection of mycotoxins by advanced and emerging analytical methods: A review. *Food Sci. Nutr.* **2020**, *8*, 2183–2204. https://doi.org/10.1002/fsn3.1474.
59. Şenyuva, H.Z.; Gilbert, J. Immunoaffinity column clean-up techniques in food analysis: A review. *J. Chromatogr. B* **2010**, *878*, 115–132. https://doi.org/10.1016/j.jchromb.2009.05.042.
60. Sospedra, I.; Blesa, J.; Soriano, J.; Mañes, J. Use of the modified quick easy cheap effective rugged and safe sample preparation approach for the simultaneous analysis of type A- and B-trichothecenes in wheat flour. *J. Chromatogr. A* **2010**, *1217*, 1437–1440. https://doi.org/10.1016/j.chroma.2009.12.047.
61. Juan, C.; Moltó, J.; Lino, C.; Mañes, J. Determination of ochratoxin A in organic and non-organic cereals and cereal products from Spain and Portugal. *Food Chem.* **2008**, *107*, 525–530. https://doi.org/10.1016/j.foodchem.2007.08.019.
62. Soleimany, F.; Jinap, S.; Faridah, A.; Khatib, A. A UPLC–MS/MS for simultaneous determination of aflatoxins, ochratoxin A, zearalenone, DON, fumonisins, T-2 toxin and HT-2 toxin, in cereals. *Food Control* **2012**, *25*, 647–653. https://doi.org/10.1016/j.foodcont.2011.11.012.
63. Suárez-Bonnet, E.; Carvajal, M.; Méndez-Ramírez, I.; Castillo-Urueta, P.; Cortés-Eslava, J.; Gómez-Arroyo, S.; Melero-Vara, J.M. Aflatoxin (B_1, B_2, G_1, and G_2) Contamination in Rice of Mexico and Spain, from Local Sources or Imported. *J. Food Sci.* **2013**, *78*, T1822–T1829. https://doi.org/10.1111/1750-3841.12291.
64. Koesukwiwat, U.; Sanguankaew, K.; Leepipatpiboon, N. Evaluation of a modified QuEChERS method for analysis of mycotoxins in rice. *Food Chem.* **2014**, *153*, 44–51. https://doi.org/10.1016/j.foodchem.2013.12.029.
65. Nazari, F.; Sulyok, M.; Yazdanpanah, H.; Kobarfard, F.; Krska, R. A survey of mycotoxins in domestic rice in Iran by liquid chromatography tandem mass spectrometry. *Toxicol. Mech. Methods* **2014**, *24*, 37–41. https://doi.org/10.3109/15376516.2013.844752.
66. Dong, M.; Si, W.; Jiang, K.; Nie, D.; Wu, Y.; Zhao, Z.; De Saeger, S.; Han, Z. Multi-walled carbon nanotubes as solid-phase extraction sorbents for simultaneous determination of type A trichothecenes in maize, wheat and rice by ultra-high performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2015**, *1423*, 177–182. https://doi.org/10.1016/j.chroma.2015.10.068.
67. Iqbal, S.Z.; Asi, M.R.; Hanif, U.; Zuber, M.; Jinap, S. The presence of aflatoxins and ochratoxin A in rice and rice products; and evaluation of dietary intake. *Food Chem.* **2016**, *210*, 135–140. https://doi.org/10.1016/j.foodchem.2016.04.104.
68. Al-Taher, F.; Cappozzo, J.; Zweigenbaum, J.; Lee, H.J.; Jackson, L.; Ryu, D. Detection and quantitation of mycotoxins in infant cereals in the U.S. market by LC-MS/MS using a stable isotope dilution assay. *Food Control* **2017**, *72*, 27–35. https://doi.org/10.1016/j.foodcont.2016.07.027.
69. Zhao, Z.; Yang, X.; Zhao, X.; Bai, B.; Yao, C.; Liu, N.; Wang, J.; Zhou, C. Vortex-assisted dispersive liquid-liquid microextraction for the analysis of major Aspergillus and Penicillium mycotoxins in rice wine by liquid chromatography-tandem mass spectrometry. *Food Control* **2017**, *73*, 862–868. https://doi.org/10.1016/j.foodcont.2016.09.035.
70. Katsurayama, A.M.; Martins, L.M.; Iamanaka, B.T.; Fungaro, M.H.P.; Silva, J.J.; Frisvad, J.C.; Pitt, J.I.; Taniwaki, M.H. Occurrence of Aspergillus section Flavi and aflatoxins in Brazilian rice: From field to market. *Int. J. Food Microbiol.* **2017**, *266*, 213–221. https://doi.org/10.1016/j.ijfoodmicro.2017.12.008.
71. Turner, N.W.; Bramhmbhatt, H.; Szabo-Vezse, M.; Poma, A.; Coker, R.; Piletsky, S.A. Analytical methods for determination of mycotoxins: An update (2009–2014). *Anal. Chim. Acta* **2015**, *901*, 12–33. https://doi.org/10.1016/j.aca.2015.10.013.

72. Zhou, S.; Xu, L.; Kuang, H.; Xiao, J.; Xu, C. Immunoassays for rapid mycotoxin detection: State of the art. *Analyst* **2020**, *145*, 7088–7102. https://doi.org/10.1039/d0an01408g.
73. Mateus, A.R.S.; Barros, S.; Pena, A.; Silva, A.S. Mycotoxins in Pistachios (*Pistacia vera* L.): Methods for Determination, Occurrence, Decontamination. *Toxins* **2021**, *13*, 682. https://doi.org/10.3390/toxins13100682.
74. Baeyens, W.R.G.; Schulman, S.G.; Calokerinos, A.C.; Zhao, Y.; García Campaña, A.M.; Nakashima, K.; De Keukeleire, D. Chemiluminescence-based detection: Principles and analytical applications in flowing streams and in immunoassays. *J. Pharm. Biomed. Anal.* **1998**, *17*, 941–953. https://doi.org/10.1016/s0731-7085(98)00062-4.
75. Shim, W.-B.; Mun, H.; Joung, H.-A.; Ofori, J.A.; Chung, D.-H.; Kim, M.-G. Chemiluminescence competitive aptamer assay for the detection of aflatoxin B1 in corn samples. *Food Control* **2014**, *36*, 30–35. https://doi.org/10.1016/j.foodcont.2013.07.042.
76. Krska, R.; Schubert-Ullrich, P.; Molinelli, A.; Sulyok, M.; Macdonald, S.; Crews, C. Mycotoxin analysis: An update. *Food Addit. Contam. Part A* **2008**, *25*, 152–163. https://doi.org/10.1080/02652030701765723.
77. Ren, Y.; Zhang, Y.; Shao, S.; Cai, Z.; Feng, L.; Pan, H.; Wang, Z. Simultaneous determination of multi-component mycotoxin contaminants in foods and feeds by ultra-performance liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* **2007**, *1143*, 48–64. https://doi.org/10.1016/j.chroma.2006.12.064.
78. Manetta, A.C. *Aflatoxins: Their Measure and Analysis*; Department of Food and Feed Science, University of Teramo Italy 2014. https://doi.org/10.5772/23965.
79. Kok, W. Derivatization reactions for the determination of aflatoxins by liquid chromatography with fluorescence detection. *J. Chromatogr. B Biomed. Sci. Appl.* **1994**, *659*, 127–137. https://doi.org/10.1016/0378-4347(94)00152-9.
80. Carballo, D.; Moltó, J.; Berrada, H.; Ferrer, E. Presence of mycotoxins in ready-to-eat food and subsequent risk assessment. *Food Chem. Toxicol.* **2018**, *121*, 558–565. https://doi.org/10.1016/j.fct.2018.09.054.
81. Ortiz, J.; Van Camp, J.; Mestdagh, F.; Donoso, S.; De Meulenaer, B. Mycotoxin co-occurrence in rice, oat flakes and wheat noodles used as staple foods in Ecuador. *Food Addit. Contam. Part A* **2013**, *30*, 2165–2176. https://doi.org/10.1080/19440049.2013.853228.
82. Bartók, T.; Tölgyesi, L.; Szekeres, A.; Varga, M.; Bartha, R.; Szécsi, .; Bartók, M.; Mesterházy, Á. Detection and characterization of twenty-eight isomers of fumonisin B1 (FB1) mycotoxin in a solid rice culture infected with Fusarium verticillioides by reversed-phase high-performance liquid chromatography/electrospray ionization time-of-flight and ion t. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 35–42. https://doi.org/10.1002/rcm.4353.
83. Zachariasova, M.; Cajka, T.; Godula, M.; Malachova, A.; Veprikova, Z.; Hajslova, J. Analysis of multiple mycotoxins in beer employing (ultra)-high-resolution mass spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 3357–3367. https://doi.org/10.1002/rcm.4746.
84. Tanaka, H.; Takino, M.; Sugita-Konishi, Y.; Tanaka, T. Development of a liquid chromatography/time-of-flight mass spectrometric method for the simultaneous determination of trichothecenes, zearalenone and aflatoxins in foodstuffs. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 1422–1428. https://doi.org/10.1002/rcm.2460.
85. Bittner, A.; Cramer, B.; Harrer, H.; Humpf, H.-U. Structure elucidation and in vitro cytotoxicity of ochratoxin α amide, a new degradation product of ochratoxin A. *Mycotoxin Res.* **2015**, *31*, 83–90. https://doi.org/10.1007/s12550-014-0218-y.
86. Warth, B.; Sulyok, M.; Fruhmann, P.; Berthiller, F.; Schuhmacher, R.; Hametner, C.; Adam, G.; Fröhlich, J.; Krska, R. Assessment of human deoxynivalenol exposure using an LC–MS/MS based biomarker method. *Toxicol. Lett.* **2012**, *211*, 85–90. https://doi.org/10.1016/j.toxlet.2012.02.023.
87. Niessen, W.M.A. Trace quantitative analysis by mass spectrometry Robert K. Boyd, Cecilia Basic, Robert A. Bethem, authors. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, R4–R6. https://doi.org/10.1016/j.jasms.2008.09.025.
88. Types of MS/MS Systems and Their Key Characteristics: SHIMADZU (Shimadzu Corporation). Available online: https://www.shimadzu.com/an/service-support/technical-support/analysis-basics/fundamental/key_characteristics.html#section5 (accessed 25 August 2022).
89. Jabbour, R.; Snyder, A. 14-Mass spectrometry-based proteomics techniques for biological identification. In *Biological Identification*; US Army Edgewood Chemical Biological Center, USA2014; pp. 370–430. https://doi.org/10.1533/9780857099167.4.370.
90. Evtugyn, G.; Subjakova, V.; Melikishvili, S.; Hianik, T. Affinity Biosensors for Detection of Mycotoxins in Food. **2018**, *85*, 263–310. https://doi.org/10.1016/bs.afnr.2018.03.003.
91. Janik, E.; Niemcewicz, M.; Podogrocki, M.; Ceremuga, M.; Gorniak, L.; Stela, M.; Bijak, M. The Existing Methods and Novel Approaches in Mycotoxins' Detection. *Molecules* **2021**, *26*, 3981. https://doi.org/10.3390/molecules26133981.
92. European Commission. Commission Implementing Regulation (EU) 2019/1715 of 30 September 2019 laying down rules for the functioning of the information management system for official controls and its system components (´the IMSOC Regulation'). *Off. J. Eur. Union* **2019**, *L261*, 37–96. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32019R1715&rid=3 (accessed on 21 July 2022).
93. How does RASFF Work. Available online: https://food.ec.europa.eu/safety/rasff-food-and-feed-safety-alerts/how-does-rasff-work_en (accessed 21 July 2022).
94. RASFF Window—Search. Available online: https://webgate.ec.europa.eu/rasff-window/screen/search?event=searchResultList (accessed 21 July 2022).
95. Trucksess, M.; Abbas, H.; Weaver, C.; Shier, W. Distribution of aflatoxins in shelling and milling fractions of naturally contaminated rice. *Food Addit. Contam. Part A* **2011**, *28*, 1076–1082. https://doi.org/10.1080/19440049.2011.576441.

96. Matumba, L.; Namaumbo, S.; Ngoma, T.; Meleke, N.; De Boevre, M.; Logrieco, A.F.; De Saeger, S. Five keys to prevention and control of mycotoxins in grains: A proposal. *Glob. Food Secur.* **2021**, *30*, 100562. https://doi.org/10.1016/j.gfs.2021.100562.
97. Oliveira, C.A.F.; Bovo, F.; Humberto, C.; Vincenzi, A.; Ravindranadha, K. *Recent Trends in Microbiological Decontamination of Aflatoxins in Foodstuffs*;Aflatoxins - Recent Advances and Future Prospects,IntechOpen 2013. https://doi.org/10.5772/51120.
98. Daou, R.; Joubrane, K.; Maroun, R.G.; Khabbaz, L.R.; Ismail, A.; El Khoury, A. Mycotoxins: Factors influencing production and control strategies. *AIMS Agric. Food* **2021**, *6*, 416–447. https://doi.org/10.3934/agrfood.2021025.
99. Mahato, D.K.; Lee, K.E.; Kamle, M.; Devi, S.; Dewangan, K.N.; Kumar, P.; Kang, S.G. Aflatoxins in Food and Feed: An Overview on Prevalence, Detection and Control Strategies. *Front. Microbiol.* **2019**, *10*, 2266. https://doi.org/10.3389/fmicb.2019.02266.
100. Sipos, P.; Peles, F.; Brassó, D.; Béri, B.; Pusztahelyi, T.; Pócsi, I.; Győri, Z. Physical and Chemical Methods for Reduction in Aflatoxin Content of Feed and Food. *Toxins* **2021**, *13*, 204. https://doi.org/10.3390/toxins13030204.
101. Hashemi, M.; Ehsani, A.; Hassani, A.; Afshari, A.; Aminzare, M.; Sahranavard, T.; Azimzadeh, Z. Phytochemical, Antibacterial, Antifungal and Antioxidant Properties of Agastache foeniculum Essential Oil. *J. Chem. Health Risks* **2017**, *7*, 95–104. https://doi.org/10.22034/JCHR.2017.544170.
102. Razzaghi-Abyaneh, M. *Aflatoxins—Recent Advances and Future Prospects*; IntechOpen: London, UK, 2013. https://doi.org/10.5772/2500.
103. Wan, J.; Zhong, S.; Schwarz, P.; Chen, B.; Rao, J. Physical properties, antifungal and mycotoxin inhibitory activities of five essential oil nanoemulsions: Impact of oil compositions and processing parameters. *Food Chem.* **2019**, *291*, 199–206. https://doi.org/10.1016/j.foodchem.2019.04.032.
104. Xing, F.; Hua, H.; Selvaraj, J.N.; Yuan, Y.; Zhao, Y.; Zhou, L.; Liu, Y. Degradation of fumonisin B1 by cinnamon essential oil. *Food Control* **2014**, *38*, 37–40. https://doi.org/10.1016/j.foodcont.2013.09.045.
105. Ponzilacqua, B.; Rottinghaus, G.; Landers, B.; Oliveira, C. Effects of medicinal herb and Brazilian traditional plant extracts on in vitro mycotoxin decontamination. *Food Control* **2019**, *100*, 24–27. https://doi.org/10.1016/j.foodcont.2019.01.009.
106. Alizadeh, A.M.; Golzan, S.A.; Mahdavi, A.; Dakhili, S.; Torki, Z.; Hosseini, H. Recent advances on the efficacy of essential oils on mycotoxin secretion and their mode of action. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 4726–4751. https://doi.org/10.1080/10408398.2021.1878102.
107. Das, S.; Singh, V.K.; Dwivedy, A.K.; Chaudhari, A.K.; Upadhyay, N.; Singh, A.; Saha, A.K.; Chaudhury, S.R.; Prakash, B.; Dubey, N.K. Assessment of chemically characterised *Myristica fragrans* essential oil against fungi contaminating stored scented rice and its mode of action as novel aflatoxin inhibitor. *Nat. Prod. Res.* **2020**, *34*, 1611–1615. https://doi.org/10.1080/14786419.2018.1519826.
108. Das, S.; Singh, V.K.; Dwivedy, A.K.; Chaudhari, A.K.; Upadhyay, N.; Singh, A.; Deepika; Dubey, N.K. Antimicrobial activity, antiaflatoxigenic potential and in situ efficacy of novel formulation comprising of Apium graveolens essential oil and its major component. *Pestic. Biochem. Physiol.* **2019**, *160*, 102–111. https://doi.org/10.1016/j.pestbp.2019.07.013.

foods

MDPI

Review

Risk of *Bacillus cereus* in Relation to Rice and Derivatives

Dolores Rodrigo *, Cristina M. Rosell and Antonio Martinez

Instituto de Agroquimica y Tecnología de Alimentos (IATA-CSIC), Paterna, 46980 Valencia, Spain; crosell@iata.csic.es (C.M.R.); amartinez@iata.csic.es (A.M.)

* Correspondence: lolesra@iata.csic.es; Tel.: +34-963900022

Abstract: Rice is a very popular food throughout the world and the basis of the diet of the citizens of many countries. It is used as a raw material for the preparation of many complex dishes in which different ingredients are involved. Rice, as a consequence of their cultivation, harvesting, and handling, is often contaminated with spores of *Bacillus cereus*, a ubiquitous microorganism found mainly in the soil. *B. cereus* can multiply under temperature conditions as low as 4 °C in foods that contain rice and have been cooked or subjected to treatments that do not produce commercial sterility. *B. cereus* produces diarrhoeal or emetic foodborne toxin when the consumer eats food in which a sufficient number of cells have grown. These circumstances mean that every year many outbreaks of intoxication or intestinal problems related to this microorganism are reported. This work is a review from the perspective of risk assessment of the risk posed by *B. cereus* to the health of consumers and of some control measures that can be used to mitigate such a risk.

Keywords: *Bacillus cereus*; rice; poisoning

Citation: Rodrigo, D.; Rosell, C.M.; Martinez, A. Risk of *Bacillus cereus* in Relation to Rice and Derivatives. *Foods* **2021**, *10*, 302. https://doi.org/10.3390/foods10020302

Academic Editor: Jean-Christophe Jacquier

Received: 23 December 2020
Accepted: 26 January 2021
Published: 2 February 2021

1. Introduction

Bacillus cereus is present in many foods due to its ubiquitous nature and has become one of the top ten responsible for many cases of food and waterborne outbreaks in humans. The demands of consumers for complex and mildly processed foods with a limited refrigerated shelf-life are driving this increase on the *B. cereus* outbreaks all over the world as has been recognized by the European Food Safety Authority EFSA and Center for Disease Control (CDC) report [1]. In 2018, *B. cereus* was involved in a total of 98 reported outbreaks among members' states of the European Union [1]. The impact of those numbers is clearer by saying that those represented the 1.9% of total outbreaks in the European Union, with 1539 people affected with 111 hospitalizations and 1 death [1]. Moreover, recent outbreaks in other countries have been also associated with this pathogen, e.g., 45 cases identified in an outbreak at a restaurant in Canberra (Australia) [2] and 200 students affected in an outbreak at a school in China [3]. Two types of food disease can be produced by *B. cereus*: diarrhoeal and emetic syndromes. Diarrhoeal illness is produced when enough *B. cereus* cells are consumed and the microorganism is implanted and grows in the small intestine producing the toxin, whereas the emetic syndrome appears when a food containing pre-formed cereulide toxin produced during *B. cereus* growth is consumed [4,5].

Generally, *B. cereus* has been associated with complex foodstuffs that include rice as ingredient; nevertheless, other rice-based products and farinaceous foods, such as pasta and noodles, can be also a vehicle for contamination and being involved in *B. cereus* intoxication [5]. This fact has promoted research on rice and carbohydrate-rich products and on improving decontamination and processing technologies that may reduce the risk of *B. cereus* poisoning. Outbreaks caused by *B. cereus* are due, in a large number of occasions, to the consumption of rice contaminated with spores or vegetative cells [6,7] (about 95% of cases of emetic disease are related to the consumption of rice [8]). Specifically, this etiological agent produced gastrointestinal diseases caused by the consumption of Chinese fried rice [9,10]. This is due to the way in which it is cooked; it is boiled in

large quantities, kept unrefrigerated for several hours, depending on consumer's demand, before being further processed (fried or heated). During this unrefrigerated storage the microorganism can grow and/or produce the emetic toxin that will not be destroyed or inactivated by a subsequent processing step [6]. These culinary practices are coherent from the logistical point of view; however, rice should be stored below 7 °C or above 63 °C, which are the limit temperatures for the germination of heat-resistant spores [11]. Those authors found that in 40 samples of local and imported rice, all of them contained *B. cereus* concentrations larger than 1100 CFU/g. Other authors [12] found that 94 out of 178 raw rice samples were contaminated with *B. cereus* in the United States. Therefore, reported results confirm how ubiquitous this microorganism, especially in rice-based foods, is. Nevertheless, the microbial loads are different depending on the rice form, being the presence of high concentrations of microorganisms more common in brown rice than in white rice, due to the processing that the cereal receives in each case. In fact, a study carried out with samples of white and husked rice reported concentrations of the pathogen around 2.5×10^1 CFU/g in husked rice and 2.5×10^3 CFU/g in brown rice [13].

Rice is the grain of herbaceous plants of the genus *Oryza* cultivated for more than 8000 years and of which about 750 million tons are produced annually mainly for human food, although the lower quality crops are destined to animal feeding [14]. It is a staple food for more than half of the world population and especially in underdeveloped countries. This cereal is presented to the consumer in different ways: whole, husked, or white depending on the treatment to which the grain was subjected. It also accepts many forms of industrial cooking and processing, steaming, parboiling, instant, ground (rice flour, pasta, and cookies) [15]. Each of them represents a different risk for the consumer depending on the subsequent treatment that consumer applies prior to consumption.

Rice, with a pH close to 7, consisting of 79% of carbohydrates, 7% protein, and 2% fat, plus vitamins and minerals, can act as an excellent growth medium for *B. cereus* once it has been cooked because it is in that moment when the humidity of the substrate reaches water activity values suitable for the growth of the microorganism. Even if the vegetative cells of *B. cereus* do not grow, they can survive 48 weeks on fresh and dry storage without loss of viability. Nevertheless, the viability of the pathogens is reduced after 16 weeks, if the storage occurs at temperatures above 45 °C with water activity around 0.78 [8].

The main problem posed by contamination with *B. cereus* of foods is the presence of heat-resistant spores that survive normal cooking temperatures for rice, or other raw materials or processed products, which is usually boiling water close to 100 °C [16]. Studies show that during normal cooking, around 20 min depending on the variety of rice, there are 2–3 decimal reductions on the initial spore load so the risk in the final product depends largely on the initial concentration of microorganisms and hygienic measures during handling, cooking, or processing [9,17]. After cooking, the remaining spores are capable of growing up to 10^7–10^9 CFU/g after 24 h at 26 or 32 °C respectively [10,11,18,19]. Spores germinate and grow depending on storage temperature; optimum growth temperatures in rice are 30–36 °C. After 10 days of storage at 8 °C, a growth of 10^4 CFU/g to 10^8 CFU/g was observed [20].

This review paper addresses the problem of *B. cereus* in rice and its derivatives under a microbiological risk assessment perspective, summarizing some control measures and improving processing technologies that could be considered to reduce the risk of the presence of the microorganism in these products and *B. cereus* toxin.

2. Hazard Description and Growth in Rice

B. cereus is a rod shaped spore-forming bacterium that belongs to the *Bacillus* genus. It is Gram-positive and motille due to flagels. *B. anthracis*, *B. cereus*, *B. mycoides*, *B. thuringiensis*, *B. pseudomycoides*, and *B. weihenstephanensis* are representative species of the 18 identified belonging to this genus [21]. Data of the genoma have shown that *B. anthracis*, *B. cereus*, and *B. thuringiensis* are very closely related; *B. thuringiensis* is an insect pathogen used in biocontrol and *B. anthracis* bacterium is responsible for anthrax [22,23]. These species show

phenotypic properties and a high level of similarity in their DNA, making biochemical identification quite difficult [22]. Different species had traditionally been differentiated by their phenotypic characteristics (e.g., shape, optimal growth temperature, resistance to acidity) but currently, other more powerful methods are being used such as digital DNA-DNA hybridization (dDDH) and/or average nucleotide identity (ANI) values [24], signature sequences (e.g., in the 16 S rRNA and cspA genes) or the presence of specific virulence factors (e.g., cytK-1 or anthrax toxin genes), and MALDI-TOF MS analysis [25] or real time PCR [26]. A recent study for Carroll [27] describes the first whole genome sequencing (WGS) characterization of isolates linked to an outbreak caused by members of the *B. cereus* group.

B. cereus is a habitual saprophyte, which is resistant to low humidity, high temperatures, dehydration, radiation, and acidity; spores are ubiquitous in the environment, inhabitant of soil, water, vegetables, and air and can be found in the soil at concentrations in the order of 10^6 CFU/g [28]. This microorganism is of interest in public health as it is considered an opportunistic pathogen that produces food toxins [16,29].

Within the *Bacillus* genus, *B. cereus* is the species most frequently associated with food outbreaks [30]. *B. cereus* is a facultative aerobic bacillus that can grow in highly variable conditions, a broad pH range between 4.5 to 9.5, at a minimum water activity for growth of 0.93, and in a broad range of temperatures from 4 °C (psychrotrophic strains) to 48 °C and at a NaCl concentrations up to 7% [31]. Although, as mentioned before, *B. cereus* tolerates a wide range of pH, the presence of 0.1% acetic acid is sufficient to inhibit the growth of the microorganism [29]. The optimum pH range from 6 to 7 and its tolerance to stress conditions due to pH, improves under anaerobically conditions [32]. Some strains of *B. cereus* are motile thanks to peritric flagella, although non-motile strains have also been described [13,33].

Although *B. cereus* have been considered as pathogenic microorganisms, researches described that some strains of this microorganism can be considered as beneficial and they have been used as animal probiotics in some formulations [34] and as growth promoters in plants [35].

The optimal growth temperature of *B. cereus ranges* between 30 and 40 °C although some strains can grow at 55 °C. Nevertheless, there are studies that have described that the strains responsible for emetic syndrome have a minimal growth temperature of 15 °C [31]. Even some strains show tolerance to lower temperatures, 4 °C, being considered psychrotrophic or psychrotolerant. Particularly, a study has suggested that *B. cereus* isolates from dairy products have adapted to those environmental conditions [36].

Vegetative cells die immediately below pH 4.3 in relation to previous exposure to acids in the environment before being ingested, despite the fact that some strains show great tolerance to gastric acids [37]. That could be the reason why the most frequent disease produced by the consumption of rice at neutral pH is the emetic syndrome, because at that pH, the microorganism lacks the necessary resistance against gastric acids to pass the intestinal tract and grows in the intestine producing toxins for emetic syndrome. By contrast, the spores have great tolerance to pH, being their viability from pH 1 to 9 [37]. In those conditions, when spores are ingested with the food, they can germinate in the small intestine and produce the diarrhoeal toxin.

Growth of *B. cereus* is optimal in the presence of oxygen, although the microorganism can grow anaerobically, but toxin production is undetectable in this environment [31]. Regarding the water activity (a_w), for vegetative cells, it should be in the range of 0.912 to 0.950 for growth [38].

Rice derivatives (boiled, fried), due to its composition and chemical characteristics, represent an excellent growth medium for bacteria and can support the growth of *B. cereus* at different temperature conditions. *B. cereus* spores can survive perfectly in the dehydrated rice, without loss of viability for at least 48 weeks of storage. However, some loss of viability has been observed if it is stored at 45 °C and water activity of 0.78 [39].

B. cereus spores have the ability to survive the cooking treatments commonly given to rice. There is high heterogeneity in the thermal resistance of the spores. Some authors [40] indicated decimal reduction (D) values between 0.94 and 11 min at 95 °C and 0.22 and 2.5 min at 100 °C in double-distilled water. Another study reported a D value of approximately 3.5 min for spores in rice at 97.8 °C [41]. With those data, and considering a standard cooking process for rice, for example 100 °C for 20 min, it is clear that there will be some level of reduction in the number of spores in the food, but it will not be enough to ensure the food safety of the food for this microorganism.

The spores that remain alive after a long-term storage of cooked rice, even at low temperatures, germinate and some degree of growth of *B. cereus* will take place. Ultee et al. [42] indicated that *B. cereus* reached levels of 10^4 to 10^8 in ten days when the product was stored at 8 °C.

3. *B. cereus* Characterization Included Dose-Response Relationship

B. cereus produces two types of illness, the emetic and diarrhoeal, depending on the context at which it grows. The diarrheal syndrome is produced as a consequence of the ingestion of a large number of vegetative cells or spores that pass the stomach barrier, during their growth in the small intestine [43]. The emetic toxin "cereulide" is a cyclic peptide, produced during the growth of *B. cereus* in the food itself, when the conditions of pH, water activity, and temperature are suitable [44]. It has a strongly hydrophobic character, therefore, to cause food poisoning, it must be attached to the target cells attached or dissolved in vehicles found in food [44]. This type of food intoxication is most often associated with the ingestion of cereal products, especially rice [45,46].

Agata et al. [47] studied the growth and emetic toxin production of *B. cereus* in cooked rice. They stored the cooked rice at different temperatures, and results indicated that the higher the temperature, the faster the growth of the microorganism and the sooner the toxin was produced. The production of cereulide was strongly correlated with the growth of bacteria in boiled rice. The growth and toxin levels at 30 °C for 24 h were similar for boiled and fried rice. In the past, studies carried out with human volunteers [48–50] found a weak significance for symptoms when *B. cereus* cells were ingested.

A human dose response relationship has not been described for either the emetic or diarrhoeal toxin produced by *B. cereus*. Epidemiological evidence suggests that the majority of outbreaks worldwide due to *B. cereus* have been associated with concentrations higher than 10^5 CFU/g in implicated foods [51–53].

3.1. Diarrhoeal Illness

The diarrhoeal syndrome is an example of a toxic infection. It is produced by enterotoxins. In the literature, the value quoted for the minimum infective dose for the diarrheal illness caused by *B. cereus* is generally higher than 10^5 cells per gram [51–53]. Kramer and Gilbert [6] stated that the levels of *B. cereus* recovered from foods implicated in outbreaks of the diarrhoeal-type illness have always been within the range 5×10^5 to 9.5×10^8 CFU/g. The symptoms of *B. cereus* diarrheal type food intoxication include abdominal pain, watery diarrheal, rectal tenesmus, moderate nausea that may accompany diarrheal, seldom vomiting, and no fever [43]. Symptoms develop within 6–15 h and can persist for 24 h. This syndrome is rather mild and tends to mimic the symptoms of *Clostridium perfringens* food poisoning [54].

3.2. Emetic Illness

The emetic illness, where toxin is pre-formed in the food, requires a high cell concentration (10^5 to 10^8 CFU/g) to produce clinically significant amounts of toxin [54]. Heating foods before consumption might remove vegetative cells of *B. cereus* but it will not destroy the heat-stable emetic toxin. Infective dose, in the case of emetic illness, is not relevant, since the disease is intoxication dependent on the amount of toxin ingested. In an outbreak in Finland, a concentration of 1.6 μg/g of emetic toxin was found in the food, assuming that

300 g of food was consumed; the toxic dose could be as high as 450 µg/g [30]. In another outbreak in the Netherlands, concentrations of 0.03–13.3 µg/g of food were found [54]. The signs of *B. cereus* emetic type food poisoning include nausea, vomiting, and headaches, abdominal cramps, and/or diarrheal. The incubation period was estimated on 1 to 5 h after the consumption of food containing cereulide-heat-and gastric acid-resistant peptide. The symptoms of this illness mimic those of *Staphylococcus aureus* food poisoning [54].

4. Evaluation of Exposition to *B. cereus* in Rice

In general, foods are contaminated with *B. cereus* spores by soil. The number of cells in soil can range from 10^3 to 10^5 spores of *B. cereus* per gram [36,55]. Spores of *B. cereus* can develop biofilms due to its adhesive properties; in consequence, foods can be contaminated during processing when circulating by pipes, surfaces, or belts [56]. This microorganism frequently appears as a spore in ready-to-eat foods since the vegetative cells usually are destroyed by the thermal processes (cooking or frying). Storage of processed products without refrigeration or under temperature abuse, or the use of raw materials in complex foods allowing *B. cereus* spores to germinate and grow can represent a risk for consumers [34].

As for the development of new preservation technologies including combined processing (hurdle technologies) such as high hydrostatic pressures, pulsed electric fields, cooked chilled foods, among others, *B. cereus* has become an emerging risk, since these processes do not eliminate the spores and, in some circumstances, produce damaged vegetative cells that can grow at temperatures of the order of 10 °C or even lower.

Restaurants or catering facilities are the most frequent places where intoxication occurred. The main responsible for *B. cereus* proliferation in foods prepared in those facilities leading to poisoning were attributed, in many cases, to inaccurate refrigeration temperature and/or the delay before preparation and consumption of dishes [34].

Studies carried out on raw rice indicate that *B. cereus* spores are frequently isolated from this food, due to its ubiquity in nature. In fact, a prevalence of 100% was observed in 2010 in Argentina [57]. Likewise, in Colombia 244 samples of foods containing rice were analysed in different regions of the country and results showed that 11.92% of those foods have concentrations higher than 10^4 CFU/g, concentrations that are considered of high risk [58].

Meals containing rice can also be a source of *B. cereus*. Studies carried out in restaurants of the United States and United Kingdom, where rice dishes were prepared, revealed that contamination can also occur after cooking, particularly through cross-contamination with spatulas used to mix rice during the cooking process [59]. In the UK, studies indicate that small restaurants pose a higher risk than large chain restaurants, owing to the poor training in hygienic practices, as well as the preparation of rice too early before being served [60].

Therefore, it is inevitable that, *B. cereus* due to its ubiquity, will be present in many raw materials. The contamination of the food during processing also requires the application of good hygienic practices and appropriate hygienic design of equipment as additional measures to control the contamination of products.

5. Control Measures

Control of *B. cereus* in rice and derivatives can be carried out at three different levels. First, there are the hygienic measures; second, the preservation processes in the production chain that would have the mission of destroying the microorganism and its spores; and third, control measures to slow down or inhibit the growth [61]. Table 1 summarizes the most important control measures described in literature.

Table 1. Some control measures for *Bacillus cereus* spores or vegetative cells.

Control Measure	Procedure	Treatment/Effect on *B. cereus*	Reference
Control initial microbial load	Use of sodium hypochlorite and weak acids on equipment	100 ppm sodium hypochlorite Weak acids at 30–40 °C for 20–30 min	[34]
Inactivation	Heat treatment	D-value (90 °C) 3.99–45 min 70 °C for 12 s, 6 log reduction (vegetative cells) 105 °C 36 s, 6 log reduction (spores)	[40,62]
	High Hydrostatic Pressure (HPP)	More than 1000 MPa	[63]
	Combined treatments	Mild heat and High Hydrostatic Pressure, between 100 and 600 MPa at 30 and 60 °C, 6 log inactivation	[64,65]
		Olive powder 2.5% and High Hydrostatic Pressure 500 MPa had additive effect	[66]
	Cold Plasma (CAP)	1.62–2.96 log CFU/mL reductions Plasma-activated water combined with mild heat, 1.5–2.12 log CFU/g reductions	[67–69]
Growth limitation or inhibition	Cold storage	Below 4 °C	[65,70,71]
		Carbon dioxide concentration higher than 40% can prevent growth of *B. cereus* stored at temperature lower than 8 °C	[72,73]
	Antimicrobials	Nisin, 500 IU/g	[74]
		Enterocin AS-48, 20–35 µg/mL	[5]
		Chitosan, 2.5% (*w*/*v*)	[75]
		Olive powder, 2.5% (*w*/*v*)	[76]

The cleaning of equipment or machines where rice circulates in the industry is the first barrier to be applied to prevent the growth of the microorganism in surfaces or pipelines and the contamination of the rice by *B. cereus*. Its spores have the ability to adhere to stainless steel surfaces of industrial equipment; this favours the growth of the microorganism on these surfaces. The use of sodium hypochlorite and weak acids is recommended on pipes and other surfaces [34]. A hygienic design of the equipment is important to avoid dead areas where the microorganism's spores can adhere and germinate.

The second barrier relies on the use of preservation procedures in the rice production chain that are capable of destroying vegetative cells and, where appropriate, bacterial spores. Heat treatment is the most common process for destroying spores and vegetative cells of microorganisms. However, *B. cereus* has a highly variable thermal resistance; in consequence, it is difficult to establish consistent pasteurization or cooking conditions. Fernandez et al. [40] reported for bacterial spores D-values at 90 °C between 3.99 min and 45 min for two strains of *B. cereus* isolated from vegetables. This difficulty is increased considering the great difference in thermal resistance between vegetative cells and spores. Byrne et al. [62] indicated that the *D*-values of *B. cereus* suggest that a mild cook of 70 °C for 12 s would achieve a 6 log reduction of *B. cereus* vegetative cells, while the equivalent reduction of *B. cereus* spores would be achieved after heating for 36 s at 105 °C in pork luncheon meat. Spores isolated from vegetables showed a $D_{105\,^{\circ}C}$ value of 0.63 min in reference substrate (pH 7) [40]. This means that a $F_{105\,^{\circ}C}$ value of 3.8 min is necessary to achieve a reduction of around 6 log; it should be taken into account that the normal cooking process of rice is carried out between 80 and 90 °C. Considering heat resistance data for very high heat resistant spores [40], heating food above 105 °C will be enough to kill *B. cereus* and protect the food from spoilage; nevertheless, only commercial sterilization ensures complete inactivation of *B. cereus* spores. Cooking, mild heat treatments, or regular

pasteurization do not inactivate all *B. cereus* spores [36]. Mild heating processed such as indicated above can instead activate spores for germination and subsequent vegetative cell growth.

Several non-thermal technologies have been used to inactivate *B. cereus* spores, but their effect was variable. At ambient temperature, a pressure higher than 1000 MPa is required for inactivation bacterial spores [63]; in consequence, no *B. cereus* spores present in a rice dish can be inactivated at the lower ordinary industrial pressure treatments (600 MPa or less). Combined treatments can help in destroying the bacterial spores. Van Opstal et al. [64] studied the inactivation of *B. cereus* spores in milk by mild pressure and heat treatments. Results indicated that all strains were reduced more than 6 logs by the two-step treatment consisting on 30 min at 200 MPa/45 °C and 10 min of cooking at 60 °C. Pina-Perez et al. [65] reported that high hydrostatic pressure and natural antimicrobials were synergic against *B. cereus* vegetative cells in a mixture of liquid whole egg and skim milk.

Marco et al. [66] studied the joint effect of the antimicrobial olive powder and high hydrostatic pressure against *B. cereus* spores in a control substrate. The authors concluded that olive powder had an additive effect to the high hydrostatic pressure processing with storage temperature and could act as an additional control measure preventing the growth of microorganisms on products pasteurised by high hydrostatic pressure technologies or reducing the potential growth in the case of cold-chain break during the shelf life. Another non-thermal technology regarded as an effective decontamination method for rice raw material after the crop harvesting is the Cold Plasma [67]. Baia et al. [68] have studied the plasma technology to inactivate *B. cereus* spores; they achieved between 1.62–2.96 log CFU/mL reductions. Liao et al. (2020) [69] studied the application of plasma-activated water combined with mild heat for the decontamination of *B. cereus* spores in rice. The treatments achieved 1.54 and 2.12 log CFU/g reductions of *B. cereus* spores in rice after 60 min exposure.

The third control measure for *B. cereus* relies on avoiding or diminishing bacterial growth. The incidence of the *B. cereus* disease is linked to the food storage temperatures and the storage time before it is finally served. Freezing or cold storage of rice-based meals (temperature lower than 4 °C) is an important strategy to control *B. cereus* [61]. Growth of *B. cereus* can be reduced by increasing the generation time, increasing doubling times or the lag phase under refrigeration storage [70,71]. According to those studies, it appears that the main control measure avoiding growth of *B. cereus* in foods is the refrigeration at temperatures below 4 °C. Refrigeration can be combined with other methods to prevent microorganism's growth. Modified atmosphere package, a carbon dioxide concentration higher than 40%, can prevent growth of *B. cereus* stored at a temperature lower than 8 °C [72,73]. At the same time, according to Andersson et al. [77], when the storage temperature was raised from 6 °C to 8 °C, growth of *B. cereus* was apparent, but after slight pH or water activity reductions, the growth of *B. cereus* was controlled at refrigeration temperatures higher than 4 °C [78]. Some food additives can be used alone or combined with other control measures against *B. cereus* in rice derivatives. Some bacteriocins such as nisin can inactivate *B. cereus* vegetative cells while essential oils-based antimicrobials such as carvacrol showed a limited effect [74,79,80]. Grande et al. [5] used enterocin AS-48 to inhibit toxicogenic *B. cereus* in rice-based foods. Inactivation of endospores was achieved by heating for 1 min at 90 °C in boiled rice or at 95 °C in rice-based gruel. Fernandes et al. [75] studied the antibacterial effects of chitosan on *B. cereus.* The use of chito-oligosaccharides alone against *B. cereus* spores was not enough to destroy a large number of cells. Ferrer et al. [76] concluded that olive powder could be used as an additional control measure in the case of cold chain break due to its effects on the lag phase *of B. cereus* vegetative cells.

In consequence, for full control of *B. cereus* concentration, it is essential to have a low initial concentration of *B. cereus* in raw materials and an adequate design of processing equipment. That should be followed by an effect preservation method and by effective cooling procedures to fast cool heat-treated foods, and storing the product below 4 °C.

Those procedures will control the concentration of B. cereus up to acceptable levels for food safety.

6. Conclusions

B. cereus will grow in most foods under favourable pH (4.5 to 9.5), water activities (>0.93), and temperatures from 4 to 48 °C. Due to its ubiquity, its spores contaminate practically all categories of foods, rice and pasta meals being the most important source of *B. cereus* spores causing intoxication. Those spores have the ability to survive the treatments commonly given to rice and other carbohydrate-rich products. Rice cooking, the mild heat applications on rice refrigerated processed foods, or regular pasteurization, as well as many non-thermal technologies, do not inactivate all *B. cereus* spores. Only the commercial sterilization can assure the complete inactivation of spores. However, it is not always possible to provide a sterilization process to such foods, since in restaurants or in collective food preparation, sterilization is not used in the production of meals. Moreover, it is also necessary to consider the detrimental effect on the nutritional or sensory properties of sterilized foods.

The *B. cereus* concentration that consumers will face will depend on raw material contamination and preservation or processing technology, but is the multiplication of *B. cereus* in foods stored under abuse refrigeration temperature that is the main contributor to the risk for human health. Rapid cooling and subsequent refrigeration storage of heat treated foods is critical and should be carefully controlled to avoid the growth of vegetative cells during the cooling phase. Moreover, to complement refrigeration with slight reductions in pH or water activity of high-carbohydrate meals will prevent multiplication of *B. cereus* at refrigeration temperatures between 4 to 8 °C.

Author Contributions: Conceptualization: A.M. and D.R.; methodology, A.M.; writing—original draft preparation, A.M.; review and editing, C.M.R. and D.R.; supervision, A.M.; funding acquisition, C.M.R., D.R. and A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by EU PRIMA program through the project "Tracing rice and valorizing side streams along mediterranean blockchain (TRACE-RICE) and through the Spanish Ministry of Science through project AGL2017-86840-C2-2-R, and to FEDER funds from EU.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: We want to thank TRACE-RICE project, Reference number AMD-1934-1 the support for this article.

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

References

1. EFSA; ECDC. The European Union One Health 2018 Zoonoses Report. Available online: https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.5926 (accessed on 29 January 2021).
2. Thirkell, C.E.; Sloan-Gardner, T.S.; Kaczmarek, M.C.; Polkinghorne, B.G. An outbreak of *Bacillus cereus* toxin-mediated emetic and diarrhoeal syndromes at a restaurant in Canberra, Australia 2018. *Commun. Dis. Intell.* **2019**, *43*, 1–9. [CrossRef] [PubMed]
3. Chen, D.; Li, Y.; Lv, J.; Liu, X.; Gao, P.; Zhen, G.; Zhang, W.; Wu, D.; Jing, H.; Li, Y.; et al. A foodborne outbreak of gastroenteritis caused by Norovirus and *Bacillus cereus* at a university in the Shunyi District of Beijing, China 2018: A retrospective cohort study. *BMC Infect. Dis.* **2019**, *19*, 910. [CrossRef] [PubMed]
4. Martin, D.W.; Gary, C.B.; Kaarin, E.G.; Michael, W.P. Risk presented to minimally processed chilled foods by psychrotrophic Bacillus cereus. *Trends Food Sci. Technol.* **2019**, *93*, 94–105.
5. Grande, M.J.; Lucas, R.; Abriouel, H.; Valdivia, E.; Ben Omar, N.; Maqueda, M.; Martínez-Bueno, M.; Martínez-Cañamero, M.; Galvez, A. Inhibition of toxicogenic *Bacillus cereus* in rice-based foods by enterocin AS-48. *Int. J. Food Microbiol.* **2006**, *106*, 185–194. [CrossRef] [PubMed]
6. Kramer, J.M.; Gilbert, R.J. Bacillus cereus and other Bacillus species. In *Foodborne Bacterial Pathogens*; Marcel Dekker Inc.: New York, NY, USA, 1989.
7. Navaneethan, Y.; Effarizah, M.E. Prevalence, toxigenic profiles, multidrug resistance, and biofilm formation of *Bacillus cereus* isolated from ready-to eat cooked rice in Penang, Malaysia. *Food Control* **2021**, *121*, 107553. [CrossRef]

8. Hocking, A.D.; Ailsa, D. Bacillus Cereus and Other Bacillus Species. In *Foodborne Microorganisms of Public Health Significance*, 6th ed.; Hocking, A.D., Ed.; Australian Institute of Food Science and Technology Inc.: North Ryde, Australia, 2003.
9. Little, C.L.; Barnes, J.; Mitchell, R.T. Microbiological quality of take-away cooked rice and chicken sandwiches: Effectiveness of food hygiene training of the management. *Commun. Dissease Public Health* **2002**, *5*, 289–298.
10. Lake, R.; Hudson, A.; Cressey, P. Risk profile of *Bacillus* spp. in rice. *N. Z. Food Saf. Auth.* **2004**. Available online: https://www.mpi.govt.nz/dmsdocument/26138/direct (accessed on 29 January 2021).
11. Maurice, B.L.; Tahar, A.S.; Shze, T.P.; Valarie, S.; Jamie, F.A.; Hashim, H.F.; Apun, K.; Radu, S. Enumeration and molecular detection of *Bacillus cereus* in local indigenous and imported rice grains. *Agric. Food Secur.* **2016**, *5*, 1–5. [CrossRef]
12. Ankolekar, C.; Rahmati, T.; Labbé, R.G. Detection of toxigenic *Bacillus cereus* and *Bacillus thuringiensis* spores in U.S. rice. *Int. J. Food Microbiol.* **2009**, *128*, 460–466. [CrossRef]
13. Sarrías, J.A.; Valero, M.; Salmerón, M.C. Elimination of *Bacillus cereus* contamination in raw rice by electron beam irradiation. *Food Microbiol.* **2003**, *20*, 327–332. [CrossRef]
14. Muthayya, S.; Sugimoto, J.D.; Montgomery, S.; Maberly, G.F. An overview of global rice production, supply, trade, and consumption. *Ann. N. Y. Acad. Sci.* **2014**, *1324*, 7–14. [CrossRef]
15. Luh, B.S. Rice production. In *Cereals Processing Technology*; Owens, G., Ed.; Woodhead Publishing Limited: Cambridge, UK, 2001; pp. 79–107.
16. Gilbert, R.J.; Stringer, M.F.; Peace, T.C. The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. *J. Hyg. Camb.* **1974**, *73*, 433–444. [CrossRef] [PubMed]
17. Shelef, L.A.; Liang, P. Antibacterial Effects of Butylated Hydroxyanisole (BHA) against *Bacillus* species. *J. Food Sci.* **1982**, *47*, 796–799. [CrossRef]
18. Yu, S.; Yu, P.; Wang, J.; Li, C.; Guo, H.; Liu, C.; Kong, L.; Yu, L.; Wu, S.; Lei, T.; et al. A Study on Prevalence and Characterization of *Bacillus cereus* in Ready-to-Eat Foods in China. *Front. Microbiol.* **2020**, *10*, 30–43. [CrossRef] [PubMed]
19. Jessim, A.I.; Fakhry, S.S.; Alwash, S.J. Detection and Determination of *Bacillus cereus* in Cooked Rice and Some Types of Spices with Ribosomal 16SrRNA gene Selected from Iraqi Public Restaurants. *Int. J. Bio-Resour. Stress Manag.* **2017**, *8*, 382–387. [CrossRef]
20. Messelhäußer, U.; Ehling-Schulz, M. *Bacillus cereus*—a Multifaceted Opportunistic Pathogen. *Curr. Clin. Microbiol. Rep.* **2018**, *5*, 120–125. [CrossRef]
21. Rajkowski, K.T.; Bennett, R.W. Bacillus cereus. In *International Handbook of Foodborne Pathogens*; Miliotis, M.D., Bier, J.W., Eds.; Marcel Dekker: New York, NY, USA, 2003; Chapter 3; pp. 27–39.
22. Martinez, J.; Sanchez, G.; Garay, E.; Aznar, R. valuation of phenotypic and PCR-based approaches for routine analysis of *Bacillus cereus* group foodborne isolates. *Antonie Leeuwenhoek* **2011**, *99*, 697–709. [CrossRef]
23. Vilas-Boas, G.; Peruca, A. Biology and taxonomy of Bacillus cereus, Bacillus antrhracis and Bacillus thuringiensis. *Can. J. Microbiol.* **2007**, *53*, 673–687. [CrossRef]
24. Liu, Y.; Du, J.; Lai, Q.L.; Zeng, R.Y.; Ye, D.Z.; Xu, J.; Zongze, S. Proposal of nine novel species of the Bacillus cereus group. *Int. J. Syst. Evol. Microbiol,* **2017**, *67*, 2499–2508. [CrossRef]
25. Takahashi, N.; Nagai, S.; Fujita, A.; Ido, Y.; Kato, K.; Saito, A.; Moriya, Y.; Tomimatsu, Y.; Kaneta, N.; Tsujimoto, Y.; et al. Discrimination of psychrotolerant *Bacillus cereus* group based on MALDI-TOF MS analysis of ribosomal subunit proteins. *Food Microbiol.* **2020**, *91*, 103542. [CrossRef]
26. Frentzel, H.; Kelner-Burgos, Y.; Deneke, C. Evaluation of a real-time PCR assay for the differentiation of *Bacillus cereus* group species. *Food Control.* **2021**, *120*, 107530. [CrossRef]
27. Carroll, L.M.; Wiedmann, M.; Mukherjee, M.; Nicholas, D.C.; Mingle, L.A.; Dumas, N.B.; Cole, J.A.; Kovac, J. Characterization of Emetic and Diarrheal *Bacillus cereus* Strains From a 2016 Foodborne Outbreak Using Whole-Genome Sequencing: Addressing the Microbiological, Epidemiological, and Bioinformatic Challenges. *Front. Microbiol.* **2019**, *10*, 144. [CrossRef] [PubMed]
28. Hendriksen, N.B.; Hansen, B.M.; Johansen, J.E. Occurrence and pathogenic potential of *Bacillus cereus* group bacteria in a sandy loam. *Antonie Leeuwenhoek* **2006**, *89*, 239–249. [CrossRef] [PubMed]
29. Sánchez, J.; Correa, M.; Castañeda-Sandoval, L.M. *Bacillus cereus* an important pathogen the microbiological control of food. *Rev. Fac. Nac. Salud Publica* **2016**, *34*, 230–242.
30. Pirhonen, T.; Anderson, M.; Jääskeläinen, E.; Salkinoja-Salonen, M.; Honkanen, T.; Johansson, T.M. Biochemical an toxinc diversity of *Bacillus cereus* in a pasta and meat dish associated with a food-poisoning case. *Food Microbiol.* **2005**, *22*, 87–91. [CrossRef]
31. Bottone, E. *Bacillus cereus*, a volatile human pathogen. *Clin. Microbiol. Rev.* **2010**, *23*, 382–398. [CrossRef]
32. Gil-Turnes, C.; Freitas dos Santos, A.; Weykamp da Cruz, F. Properties of the *Bacillus cereus* strain used in probiotic CenBiot. *Rev. Microbiol.* **1999**, *30*, 11–15. [CrossRef]
33. Finlay, W.J.; Logan, N.A.; Sutherland, A.D. *Bacillus cereus* emetic toxin production in cooked rice. *Food Microbiol.* **2002**, *19*, 431–439. [CrossRef]
34. EFSA. Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs. *EFSA J.* **2005**, *175*, 1–48.
35. Halverson, L.; Handelsman, J. Enhancement of Soybean Nodulation by *Bacillus cereus* UW85 in the field and in growth chamber. *Appl. Environ. Microbiol.* **1991**, *57*, 2767–2770. [CrossRef]
36. Kotiranta, A.; Lounatmaa, K.; Haapasalo, M. Epidemiology and pathogenesis of *Bacillus cereus* infections. *Microb. Infect.* **2000**, *2*, 189–198. [CrossRef]

37. Clavel, T.; Carlin, F.; Lairon, D.; Nguyen-The, C.; Schmitt, P. Survival of *Bacillus cereus* spores and vegetative cells in acid media simulating human stomach. *J. Appl. Microbiol.* **2004**, *97*, 214–219. [CrossRef] [PubMed]
38. Mols, M.; Pier, I.; Zwietering, M.H.; Abee, T. The impact of oxygen availability on stress survival and radical formation of *Bacillus cereus*. *Int. J. Food Microbiol.* **2009**, *135*, 303–311. [CrossRef] [PubMed]
39. Jaquette, C.B.; Beuchat, L.R. Survival and growth of psychrotrophic Bacillus cereus in dry and reconstituted infant rice cereal. *J. Food Prot.* **1998**, *61*, 1629–1635. [CrossRef] [PubMed]
40. Fernandez, A.; Ocio, M.J.; Fernandez, P.S.; Rodrigo, M.; Martinez, A. Application of non-linear regression analysis to the estimation of kinetic parameters for two enterotoxigenic strains of *Bacillus cereus* spores. *Food Microbiol.* **1999**, *16*, 607–613. [CrossRef]
41. Penna, T.C.V.; Moraes, D.A. The influence of nisin on the thermal resistance of *Bacillus cereus*. *J. Food Prot.* **2002**, *65*, 415–418. [CrossRef]
42. Ultee, A.; Slump, R.A.; Steging, G.; Smid; E. J. Antimicrobial activity of carvacrol toward *Bacillus cereus* on rice. *J. Food Prot.* **2000**, *63*, 620–624. [CrossRef]
43. Granum, P.E.; Lund, T. *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiol. Lett.* **1997**, *157*, 223–228. [CrossRef]
44. Agata, N.; Ohta, M.; Mori, M.; Isobe, M. A novel dodecadepsipeptide, cerulide, is an emetic toxin of *Bacillus cereus*. *FEMS Microbiol. Lett.* **1995**, *129*, 17–20.
45. Berthold-Pluta, A.; Pluta, A.; Garbowska, M. The efect of selected factors on the survival of *Bacillus cereus* in the human gastrointestinal tract. *Microb. Pathog.* **2015**, *82*, 7–14. [CrossRef]
46. Ehling-Schulz, M.; Frenzel, E.; Gohar, M. Food-bacteria interplay: Pathometabolism of emetic *Bacillus cereus*. *Front. Microbiol.* **2015**, *6*, 704. [CrossRef]
47. Agata, N.; Ohta, M.; Yokoyama. K. Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. *Int. J. Food Microbiol.* **2002**, *73*, 23–27. [CrossRef]
48. Hauge, S. Food poisoning caused by aerobic spore forming bacilli. *J. Appl. Bacteriol.* **1955**, *18*, 591–595. [CrossRef]
49. Dack, G.M.; Sugiyama, H.; Owens, O.J.; Kirsner, J.B. Failure to produce illness in human volunteers fed *Bacillus cereus* and *Clostridium Perfringens*. *J. Infect. Dis.* **1954**, *94*, 34–41. [CrossRef] [PubMed]
50. Langeveld, L.P.M.; van Sponsen, W.A.; van Beresteijn, E.C.H.; Notermans, S. Consumption by healthy adults of pasteurised milk with a high concentration of *Bacillus cereus*: A double-blind study. *J. Food Prot.* **1996**, *59*, 723–726. [CrossRef] [PubMed]
51. Schoeni, J.L.; Lee Wong, A.C. *Bacillus cereus* food poisoning and its toxins. *J. Food Prot.* **2005**, *68*, 636–648. [CrossRef]
52. Notermans, S.; Dufrenne, J.; Teunis, P.; Beumer, R.; te Giffel, M.; Peeters Weem, P. A risk assessment study of Bacillus cereus present in pasterurized milk. *Food Microbiol.* **1997**, *14*, 143–151. [CrossRef]
53. Granum, P.E.; Baird-Parker, T.C. Bacillus species. In *The Microbiological Safety of Quality of Food*; Lund, B.M., Baird-Parker, T.C., Gould, G.W., Eds.; Aspen: Gaithersburg, MD, USA, 2000; Volume 2, pp. 1029–1039.
54. Tajkarimi, M. *Bacillus cereus. PHR 250. Materials from Maha Hajmeer*; 2007; 6p. Available online: https://www.cdfa.ca.gov/ahfss/Animal_Health/PHR250/2007/25007BcerMH__2_.pdf (accessed on 29 January 2021).
55. Guinebretiere, M.; Girardin, H.; Dargaingnaratz, C.; Carlin, F.; Nguyen-The, C. Contamination flows for *B. cereus* and spore-forming aerobic bacteria in a cooked, pasteurized and chilled zucchini purée processing line. *Int. J. Food Proct.* **2003**, *82*, 223–232. [CrossRef]
56. Jullien, C.; Benezech, T.; Carpertier, B.; Lebret, V.; Faille, C. Identification of surface characteristics relevant to the hygienic status of stainless steel for the food industry. *J. Food Eng.* **2003**, *56*, 77–87.
57. Fangio, M.; Roura, S.; Fritz, R. Isolation and Identification of *Bacillus* spp. and Related Genera from different Starchy Foods. *J. Food Sci.* **2010**, *75*, M218–M221. [CrossRef]
58. UERIA Perfil de Riesgo Bacillus cereus en Alimentos Listos Para su Consumo no Industrializados; Ministerio de la Protección Social Unidad de Evaluación de Riesgos para la Inocuidad de los Alimentos UERIA Instituto Nacional de Salud INS. 2011. Available online: https://docplayer.es/14657995-Perfil-de-riesgo-bacillus-cereus-en-alimentos-listos-para-consumo-no-industrializados.html (accessed on 29 January 2021).
59. Bryan, F.L.; Bartleson, C., A.; Cristopherson, N. Hazard analysis in reference to *Bacillus cereus* of boiled and fried rice in Cantonese style restaurants. *J. Food Proct.* **1981**, *44*, 500–512. [CrossRef]
60. Nichols, G.L.; Little, C.L.; Mithani, V.; de Louvois, K. The microbiological quality of cooked rice from restaurants and take away premises in the Unided Kigdom. *J. Food Proct.* **1999**, *62*, 877–882. [CrossRef]
61. Tessi, M.A.; Arìngoli, E.E.; Pirovani, M.E.; Vincenzini, A.Z.; Sabbag, N.G.; Costa, S.C.; Garcia, C.C.; Zannier, M.S.; Silva, E.R.; Moguilevsky, M.A. Microbiological Quality and Safety of Ready-to-Eat Cooked foods from a centralized School Kitchen in Argentina. *J. Food Proct.* **2002**, *65*, 636–642. [CrossRef]
62. Byrne, B.; Dunne, G.; Bolton, D.J. Thermal inactivation of *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spores in pork luncheon roll. *Food Microbiol.* **2006**, *23*, 803–808. [CrossRef] [PubMed]
63. Smelt, J.P.P.M.; Hellemons, J.C.; Wouters, P.C.; Van Gerwen, S.J.C. Physiological and mathematical aspects in setting criteria for decontamination of foods by physical means. *Int. J. Food Microbiol.* **2002**, *78*, 57–77. [CrossRef]
64. Van Opstal, I.; Bagamboula, C.F.; Vanmuysen, S.C.M.; Wuytack, E.Y.; Michiels, C.W. Inactivation of *Bacillus cereus* spores in milk by mild pressure and heat treatments. *Int. J. Food Microbiol.* **2004**, *92*, 227–234. [CrossRef] [PubMed]

65. Pina-Perez, M.C.; Silva-Angulo, A.B.; Muguerza-Marquınez, B.; Rodrigo, D.; Martınez Lopez, A. Synergistic Effect of High Hydrostatic Pressure and Natural Antimicrobials on Inactivation Kinetics of *Bacillus cereus* in a Liquid Whole Egg and Skim Milk Mixed Beverage. *Foodborne Pathog. Dis.* **2009**, *6*, 649–656. [CrossRef]
66. Marco, A.; Ferrer, C.; Velasco, L.M.; Rodrigo, D.; Muguerza, B.; Martınez, A. Effect of olive powder and high hydrostatic pressure on the inactivation of *Bacillus cereus* spores in a reference medium. *Foodborne Pathog. Dis.* **2011**, *8*, 681–685. [CrossRef]
67. Liao, X.Y.; Muhammad, A.I.; Chen, S.G.; Hu, Y.Q.; Ye, X.Q.; Liu, D.H.; Ding, T. Bacterial spore inactivation induced by cold plasma. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 2562–2572. [CrossRef]
68. Baia, Y.; Muhammada, A.I.; Hua, Y.; Kosekic, S.; Liaoa, X.; Chena, S.; Yea, X.; Liua, D.; Dinga, T. Inactivation kinetics of *Bacillus cereus* spores by Plasma activated water (PAW). *Food Res. Int.* **2020**, *131*, 109041. [CrossRef]
69. Liao, X.Y.; Bai, Y.; Muhammad, A.I.; Liu, D.H.; Hu, Y.Q.; Ding, T. The application of plasma-activated water combined with mild heat for the decontamination of Bacillus cereus spores in rice (*Oryza sativa* L. ssp. japonica). *J. Phys. D Appl. Phys.* **2020**, *53*, 064003. [CrossRef]
70. Choma, C.; Guinebretiere, M.H.; Carlin, F.; Schmitt, P.; Velge, P.; Granum, P.E.; Nguyen-The, C. Prevalence, characterization and growth of Bacillus cereus in commercial cooked chilled foods containing vegetables. *J. Appl. Microbiol.* **2000**, *88*, 617–625. [CrossRef] [PubMed]
71. Valero, M.; Leontidis, S.; Fernández, P.; Martínez, A.; Salmerón, C. Growth of *Bacillus cereus* in natural and acidified carrot substrates over the temperature range 5–30 °C. *Food Microbiol.* **2000**, *17*, 605–612. [CrossRef]
72. Benniket, M.H.J.; Smid, E.J.; Rombouts, F.M.; Gorris, L.G.M. Growth of psychrotrophic foodborne pathogens in a solid surface model system under the influence of carbon dioxide and oxygen. *Food Microbiol.* **1995**, *12*, 509–519. [CrossRef]
73. Samapundo, S.; Everaert, H.; Wandutu, J.N.; Rajkovic, A.; Uyttendaele, M.; Devlieghere, F. The influence of headspace and dissolved oxygen level on growth and haemolytic BL enterotoxin production of a psychrotolerant *Bacillus weihenstephanensis* isolate on potato based ready-to-eat food products. *Food Microbiol.* **2011**, *28*, 298–304. [CrossRef] [PubMed]
74. Ray, B. Nisin of *Lactococus lactis* ssp lactus as a food biop'reservative. In *Food Biopreservatives of Natural Origen*; Ray, B., Daeschel, M.A., Eds.; CRC Presss: Boca Raton, FL, USA, 1992; pp. 207–264.
75. Fernandes, J.C.; Eaton, P.; Gomes, A.M.; Pintado, M.E.; Malcata, F.X. Study of the antibacterial effects of chitosans on *Bacillus cereus* (and its spores) by atomic force microscopy imaging and nanoindentation. *Ultramicroscopy* **2009**, *109*, 854–860. [CrossRef]
76. Ferrer, C.; Ramón, D.; Muguerza, B.; Marco, A.; Martínez, A. Effect of Olive Powder on the Growth and Inhibition of *Bacillus cereus*. *Foodborne Pathog. Dis.* **2009**, *6*, 33–37. [CrossRef] [PubMed]
77. Andersson, A.; Ronner, U.; Granum, P.E. What problems does the food industry have with the spore-forming pathogens *Bacillus cereus* and *Clostridium perfringens*? *Int. J. Food Microbiol.* **1995**, *28*, 145–155. [CrossRef]
78. Benedict, R.C.; Partridge, T.; Wells, D.; Buchanan, R.L. *Bacillus cereus*, aerobic growth kinetics. *J. Food Prot.* **1993**, *56*, 211–214. [CrossRef]
79. Pol, I.; Smid, E.J. Combined action of nisin and carvacrol on *Bacillus cereus* and *Listeria monocytogenes*. *Lett. Appl. Microbiol.* **1999**, *29*, 166–170. [CrossRef]
80. Periago, P.M.; Moezelaar, P. The combined effect of nisin and carvacol at different pH and temperature levels on the viability of different strains of *Bacillus cereus*. *Int. J. Food Microbiol.* **2001**, *68*, 141–148. [CrossRef]

TRACE-RICE

TRACE-RICE Consortium

Instituto Nacional de Investigação Agrária e Veterinária, I.P.

iBET Instituto de Biologia Experimental e Tecnológica

ITQB Universidade Nova de Lisboa

Casa do Arroz

ernesto morgad desde 1920

Mater Dynamics

BGI www.bgi.pt

Grupo Desarrollo

iata Instituto de Agroquímica y Tecnología de Alimentos

Alexandria University