

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Aflatoxins Occurrence in Spices

Farman Ahmed and Muhammad Asif Asghar

Abstract

A wide range of spices are used in most dishes as seasoning, colouring, texture developer, palatability or preserving food and beverages worldwide. However, the spices are produced mainly in developing countries where tropical and/or subtropical climate such as high temperature, heavy rainfall and humidity encourage fungal growth leading to increased occurrence of aflatoxins (AFs) in spices. Moreover, the inadequate implementation of good agricultural practice, good manufacturing practice and good hygienic practice in these countries are great alarming situation. AFs are considered as a carcinogenic, mutagenic, teratogenic and immunosuppressive to humans and are classified as hazardous food toxins. This chapter provides the worldwide production and regulations of spices, suitable conditions for the AFs production, worldwide occurrence of AFs, detection techniques and some aspect for the reduction of AFs in spices.

Keywords: detoxification techniques, climatic variation, potential exposure in human

1. Introduction

Nowadays, scientists are focusing on efficient control of the occurrence of xenogenous constituents in foodstuffs which might be risk for the public health. Spices native to India were grown as early as the 8th century BC in the gardens of Babylon. Spices are considered one of the valuable crops in the world due their important characteristic such as flavoring, colouring and aromatizing as well as antimicrobial and antioxidant effect [1]. Spices are extensively used as a staple crop and cultivated in tropical and sub-tropical regions.

Spices are considered as the non-leafy fragments of plant such as seed, bud, bark, fruit, rhizome or bulb. However, the leaf and flower are designated as different group known as herbs. However, all parts of a plant should be considered to be spices if they possess the aforementioned properties for meal enhancement such as its color, flavor or even texture [2]. Unfortunately, many spices are very susceptible to toxigenic fungal strains and are likely to produce aflatoxins (AFs) contamination [3–5]. Fungal growth is also exaggerated by the landform, soil natures and properties along with interactions between the micro-fungus and micro- or macro-organisms in soil. In addition, harvesting, drying, handling, packing, carrying, due to probable physical rupturing, insect damage, growth and metabolic action of fungal are also responsible to propagate the fungal proliferation. Moreover, spices purchased in loose or open packing are proved to be considerably more contaminated than spices purchased in sealed or close packing [6].

AFs are naturally occurring metabolites mostly created by *Aspergillus flavus* and *A. parasiticus*. *A. bombysis*, *A. ochraceoroseus*, *A. nomius*, and *A. pseudotamari* are

rarely AFs-producing species. From the mycological viewpoint, each strain shows different qualitative and quantitative abilities to produce AFs. For instance, only about half of *A. flavus* strains produced AFs-producing species more than 10^6 $\mu\text{g}/\text{kg}$ [7]. Aflatoxins B and G are produced by the *A. parasiticus* and more improved to a soil environment with limited spreading. Presently, 18 different types of AFs have been recognized, aflatoxin B₁, B₂, G₁ and G₂ (AFB₁, AFB₂, AFG₁ and AFG₂) is considered more toxic but AFB₁ is the most recurrently arising amongst all of them [8]. The order of chronic and acute poisonousness is AFB₁ > AFG₁ > AFB₂ > AFG₂. AFs are among the most studied mycotoxins globally. AFs are linked with several diseases such as aflatoxicosis in humans, birds, fishes and livestock domestic animals [9]. AFs are the most harmful and tremendously mutagenic mycotoxins [10]. Moreover, AFs have been designated as the first class carcinogens by the International Agency for Research on Cancer (IARC) [11]. AFB₁ is deliberated as most toxic due to its extreme hepatotoxic and hepatocarcinogenic ability. The liver is recognized as the prime target organ [12]. Furthermore, the cyclic nucleotide phosphodiesterase action in the heart, kidney, liver and brain tissues could be inhibited by AFB₁. This results in the malfunctioning of these body parts.

Due to above declared facts; many countries have enforced strict guidelines concerning AFs occurrence in food and their products [4]. In addition, spices are frequently vulnerable to AFs contamination, as reported in assessments from various states [13–15]. Spices are mainly produced in tropical climatic regions with higher range of humidity, temperature and rainfall [16]. Moreover, inadequate storage, prolonged drying periods and higher moisture contents may cause improvement of AFs in spices. The lacking of infrastructure against fungal attack on food commodities in developing countries (e.g. India, Sri Lanka and Pakistan) causes AFs problems. Limited execution of Good Hygienic Practice (GHP), Good Agricultural Practice (GAP), Good Manufacturing Practice (GMP), Good Storage Practice (GSP), improper storing and inadequate shipping could also liable to *Aspergillus* growth and proliferation the hazard of AFs contamination. The condition regarding AFs contamination in spices is well accepted [3]. Still there is a need to collect information regarding AFs in different states around the world. Consequently, this chapter provides the comprehensive facts regarding occurrence of AFs in spices.

2. Worldwide spices cultivation

During the last 5 years (2015–2019), the average production of spices was c. 602126902 tonnes and 127787137 tonnes in 2019. These spices includes anise, badian, fennel, coriander, chilies and peppers, cinnamon, cloves, garlic, ginger, nutmeg, mace, cardamoms, mustard seed, pepper (*piper* spp.), peppermint and vanilla”. Asia is considered the largest producer of spices in the world with the production share of 76.2% (197818212 tonnes in 2019). Whereas, India provides most to this segment (9508837 tonnes in 2019), followed by China (5307696 tonnes in 2019) [17]. Currently, the global spices and seasonings market is undergoing a healthy growth. Looking forward, the market is predicted to reveal a CAGR of around 4.7% during 2020–2025 [18].

3. Suitable climatic conditions for AFs production

The climate is considerably influence on the accessibility and quality of the spices. The change in climate simultaneously impacts the complex of AF-producers to change its fungal community's structure. The temperature and water activity (aw)

in the atmosphere alters by the climate which further impacts the gene appearance to produce AFs [19]. The AF-producing genes are clustered on the genome and express the main regulatory genes as transcription activator aflatoxin (*aflR*), pathway regulator aflatoxin (*aflS*) as well as structural genes such as reductase aflatoxin (*aflD*) which are subjective by the contact of temperature and aw conditions [20]. As revealed by the [21], the expression fraction of *aflR/aflS* significantly connects with the amount of AFB₁ produced. Most examinations in regions with warm climates have emphasized the occurrence of fungal species of the genus *Aspergillus* in spices. Generally, the greater contamination is found in warm, humid and even hot deserts and drought environments [22]. However, ideal situations for the AFs formation is considered as moisture content between 18 to 20%, water activity >0.82, pH 3.0 to 8.5 and ambient temperature between 12 to 40 °C (54 to 104 °F) with an optimal at 25 to 30 °C (77 to 86 °F). Nutrition aspects such as carbohydrate, nitrogen sources, zinc, phosphates and other trace metals also influence the development of AFs [23].

Country	Commodities	Maximum acceptable limits (µg/kg)	
		AFB ₁	AFB ₁ , AFB ₂ + AFG ₁ + AFG ₂
United States (FDA & FAO)	All foods	—	20
EU	Spices	5	10
Bulgaria	Spices	2	5
Croatia	Spices	30	15
Brazil	Spices	—	20
China	Spices	5	—
Czech Republic	Spices	20	—
Finland	All Spices	—	20
Indonesia	Spices powder	15	5
Brazil	Spices	20	30
India	All foods	30	—
Turkey	Spices	10	—
Iceland	All Spices	30	—
Iran	Spices	5	10
Republic of South Africa	All foods	5	10
Australia	All foods	—	5
Uruguay	All Spices	5	20
Hong Kong	All foods	—	15
Malaysia	All foods	—	35
Japan	All foods	10	—
Singapore	All foods	—	0
New Zealand	All foods	—	5
Sri Lanka	All foods	—	30
Pakistan	Selected Spices	—	30

Table 1.
 MTL as established by various countries for AFB₁ and AFs (B₁, B₂, G₁ and G₂) in spices and foods.

4. Regulations of aflatoxins in spices

Though large number of mycotoxins occur in nature however only few toxins (e.g. Aflatoxins) creates food safety and security problems. Therefore, it is necessary, to prevent dangerous outbreaks of these toxins in humans and animals, also to control them within tolerance limits assigned by international agencies. The international regulatory agencies & authorities establish maximum tolerated limit (MTL) for AFs in spices because of severe toxicity of these toxins. The MTL relating to AFs differ from country to country, as developed nations have set lower tolerance limits as compare to developing countries where these susceptible commodities are produced [24]. In addition, the MTL differ from one country to another because of different agricultural practices and climatic conditions. The Food and Agriculture Organization (FAO) has stated that nearly 100 countries have been established MTL for mycotoxins or minimum only for AFs. While 13 countries are uncertain to provide specific regulations and almost 50 countries have no regulations or no data exist [25].

As it is evident from these data, at present, a fair number of the Codex member states have fixed the maximum limits on AFs in spices. These limits range from 1 µg/kg (Honduras) to 30 µg/kg (India) [4]. The first tolerance level of 30 µg/kg for total AFs in all foods was legislated in 1965 by the USFDA. Later, it was reduced to 20 µg/kg due to the potent toxicity of AFs [26]. The European Union' Scientific Committee on Food (SCF) established the MTL in spices i.e. 5 µg/kg for AFB₁ and 10 µg/kg for total AFs [27]. In 2017, the National Standard of China has been updated by the National Food Safety Standard for Maximum Levels of Mycotoxins in Foods (GB 2761–2017) and in January 2020, the public consultation on its revision was launched. While under the National Standard, the maximum level is set at 5 µg/kg for AFB₁ in spices [4]. In India, the MTL prescribed for AFs in spices by the Food Safety and Standards Authority is 30 µg/kg. Also, MTL have also been established for AFs in different spices in many countries as listed in **Table 1**.

5. Aflatoxins detection techniques in spices

To develop effective and valid analytical methods extensive researches have been carried out for qualitative and quantitative detection or determination of AFs in spices. Generally, the determination of AFs is performed in two steps, (i) extraction or clean-up of samples and (ii) detection or quantification of AFs. The purpose for the use of different extraction and clean-up methods is to separate AFs from other matrix components and to minimize the impact of heterogeneous distribution of AFs [28]. As a result, reduce the background signal during the instrumental analysis. Conventional extraction approaches are unable to precisely & accurately analyse AFs in spices. It is because of the presence of natural colour/pigment producing background interference in HPLC Analysis results masking of toxin [29]. In addition, the complexity of natural constituents in spices matrix frequently makes it challenging to efficiently extract the AFs. Various extraction solvents are currently in use such as Methanol: Water (80:20), Acetonitrile: Water (60:40), Acetone: Water (75:25) and Methanol: Water (60:40). The reported maximum recovery was achieved by Methanol: Water (80:20) [30, 31]. However, various clean-up methods have been proposed as mentioned below:

- Liquid–Liquid Extraction (LLE)
- Solid Phase Extraction (SPE)

- Immunoaffinity Column Clean-up (IAC)
- Supercritical Fluid Extraction (SFE)
- Energy-Assisted Extraction (EAE)

To clean up AFs in foodstuffs, SPE using a silica gel column, a florisil column, or multifunctional columns has been used. Recently, immunoaffinity (IA) chromatography utilizing immunological interaction which has a high clean-up effect has been employed as well [32]. In addition, the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method is a simple and straightforward technique has also been utilized for AFs extraction [33, 34]. Furthermore, the detection methods are based on the emission and absorption characteristics such as thin layer chromatography (TLC), 2D-TLC (Two Dimensional), Enzyme linked immunosorbent assay (ELISA), Fluorometric determination, High performance liquid chromatography (HPLC), Gas chromatography (GC) and Liquid chromatography linked with tandem mass spectrometry (LC-MS-MS). Still, there are certain advantages and disadvantages linked with these developed techniques e.g. reduced sensitivity, unsatisfied accuracy, scope restriction, cost, laborious and sometimes extended analysis time [35, 36].

Thin layer chromatographic technique is considered as preliminary analytical testing method for mycotoxins. From time to time, TLC is performed for the confirmation of other methods or techniques outcomes & effectively being practiced in developing countries because of low cost. The difficulties of TLC method of analyses are low sensitivity, sometimes poor separation and unsatisfactory accuracy. Special skills are also required to observe the separated spots of compounds (toxin) on TLC cards in presence of ultraviolet or fluorescent lamps. The analyte is difficult to separate from sample matrix in this technique. Also, the poor reproducibility or repeatability in TLC because of factors e.g. sample extraction in solvents, application of extracted sample as spots on TLC card or plate and observer visualization [37]. The fluorometric system is relatively sensitive and accurate as compare to conventional spectrophotometric systems. More consideration and sensitive equipment technique is also prerequisite regarding environmental factors [38].

ELISA technique is widely applied since few years, because of its simplicity, sensitivity and fast quantification of AFs. More benefits of ELISA include simplicity of sample preparation and probably low analysis cost. However, ELISA is established on the immunological mechanism & it needed very specific monoclonal & polyclonal sera for sensitive and specific quantification of antigen. Also, it consume long incubation time, mixing & washing steps and it is unable to analyse AFs individually [39].

On the contrary to ELISA & TLC, the HPLC method has taken more attention because of its accuracy, sensitivity, specificity and reproducibility, also, the HPLC instrumental analysis is capable to measure single toxin exclusively. In other food matrices, HPLC has increased attraction where determination of AFB₁ is necessary requirement solely. HPLC determination is remarkably sensitive as compare to the TLC system and it can measure AFB₁ toxin even if less than 0.1 µg/kg [40]. Conversely, the disadvantage of HPLC is the toxins need derivatization to improve their fluorescence properties. AFs have natural fluorescence characteristics due to the rigid and conjugated molecular structure. In these circumstances, small alterations in the molecular structure may increase fluorescence characteristic significantly. Hence, prerequisite derivatization with aid of chemicals boosts their fluorescence property. For instance, AFG₂ and AFB₂ are more fluorescent in comparison with AFB₁ and AFG₁.

Several derivatization methods of AFB₁ and AFG₁ are presently in practice. The frequent techniques include (i) pre-column treatment with trifluoroacetic acid (TFA) [41], (ii) post-column derivatization with iodine [42], (iii) cyclodextrins [43] and (iv) pyridinium hydrobromide perbromide [44]. The drawbacks related to the TFA based treatment method are the longer reaction time with elevated temperature, TFA toxicity and its corrosiveness [45]. Similarly, iodine based method also does have prolonged reaction time (up to 2 min) at elevated temperatures along with chromatogram peak broadening and need of supplementary HPLC pump. Also, daily preparation of the iodine solution is not recommended because of its corrosiveness to avoid capillary blockage and draining of the reagent pump seals [46]. All of the above four methods are prolonged and linked with the some weaknesses. In these circumstances, an exciting methodology of photochemical derivatization is known as Kobra Cell™ (R-Biopharm, Rhone Ltd., UK). This derivatization does not need everyday preparation of any reagent. There is no instability or corrosiveness issues and photochemical derivatization time is 4 seconds only at ambient temperature [40]. Likewise the method with a low maintenance cost no supplementary pump is needed.

The Gas Chromatography methodology is also operated for the determination of particular AFs which are challengingly measured by HPLC. Unfortunately, this technique is time-consuming and expensive. Sometimes it is required to transform analyte into the volatile compound compared to other methods [47]. Sometimes, pre-derivatization is prerequisite for the AFs before GC injection. Thermal stability of the AFs extract is also an issue because AFs decomposes at high temperature. In addition, the GC is not widespread using for the commercial testing of AFs because of greater running cost than HPLC analysis.

Nowadays, the advanced version of Liquid Chromatography Mass Spectrometry i.e. LC/MS/MS system has achieved more attention for AFs determination. The LC/MS/MS benefits over other techniques are of extra ordinary sensitivity (lower level detection), greatly specific and the confirmation support of mass spectral fragments and identification of interfering impurities [48]. A single run of LC/MS/MS can support the quantification and determination of multiple mycotoxins [49]. Conversely, to get desire selectivity and sensitivity, there is need of additional accuracy in the sample preparation steps. The LC/MS/MS is an expensive equipment unit and it requires more skill for analytics hence it is more recommended for R & D. The extraction specific solvents for LC/MS/MS sample preparation is an additional requirement.

The variation in these approaches could be established because of the effect of matrix and matrix parameters, the intensity of AFs contamination in product. A validated method is desirable in these situations, a method which avoid maximum matrix effect and close by the tolerable limits as obligatory by the international legislative authorities.

6. Worldwide contamination of aflatoxins in spices

This section of the chapter describes the studies regarding AFs in spices over the last few years. A total of 27 studies altogether covering 19 spices were included. AFs incidence in spices differs place to place due to temperature & moisture differences, microflora and agronomics variations [31]. The worldwide occurrence of AFB₁ and total AFs with respect to each unique spice are shown in **Table 2**. In terms of AFs, studies are most often concerned with red chilli, black pepper, caraway, cinnamon, aniseed, cumin, ginger, red pepper, clove, fenugreek, coriander, cardamom, turmeric, paprika, curry, garlic and mix spices. The occurrence of total AFs in the above-mentioned spices is usually high to very high.

Country	Category of spices	n	Positive samples (%)	AFB ₁		Total AFs		Study Year	References
				Range	Mean	Range	Mean		
Pakistan	1	331	97	—	—	1.3–93.7	17.2	2006–2011	[31]
Turkey	2,3,5,6,8,9,19	93	58	0.5–52.5	20.7	—	—	2010–2011	[50]
Pakistan	1	69	67	—	—	1.2–600.0	57.5	2012	[51]
Turkey	9	42	90	—	—	0.4–86.0	17.1	2013	[52]
Turkey	1	182	82	0.2–165.0	—	—	—	2013	[53]
India	1,3	18	18	31.1–174.7	—	—	—	2013	[54]
Iran	3,9	76	54	0.88–28.6	15.5	1.4–30.2	15.5	2014	[55]
Algeria	2,3,4,5,6,7,8,9,11	36	63.9	0.1–26.5	—	—	—	2015	[5]
Iran	3,5,9,13	80	40	0.8–17.9	—	0.8–24.1	—	2015	[56]
South Africa	1,8,15,19	70	40	3.0–19.0	—	—	—	2015	[57]
Iran	3,7, 8,9,13	120	31	0.2–57.5	—	0.7–57.5	—	2015	[58]
Malaysia	1,3,5,6,7,13	25	88	0.3–28.4	7.3	0.32–31.17	8.4	2015	[59]
Pakistan	19	75	77	—	—	0.7–25.7	4.6	2015	[6]
Saudi Arabia	1	60	57	—	—	0–200	16.0	2015	[60]
USA	1	169	64	ND–94.9	4.8	—	—	2015–2016	[14]
Nigeria	1	55	93	ND–156.0	13.5	—	—		
Italy	12	45	31	LOQ–155.7	12.8	LOQ–529.1	13.9	2017	[61]
Tanzania	5,8,10,16	120	57	0.15–11.2	0.8	0.1–11.9	1.4	2017–2018	[62]
Pakistan	1,3	50	30	23.9–75.8	—	—	—	2018	[63]
Lebanon	1,2,5,6,7, 8,10,11,13,15,16,17,18,19	94	19	2.2–1118.2	193.4	2.2–1118.2	168.1	2018	[64]
Pakistan	1,3,13,19	120	100	1.0–30.4	6.54	1.5–44.3	9.7	2018	[13]

Country	Category of spices	n	Positive samples (%)	AFB ₁		Total AFs		Study Year	References
				Range	Mean	Range	Mean		
Bangladesh	1	50	75	—	—	12.0–68.7	—	2018	[65]
Iran	3,5,9,13	80	50	—	—	1.2–77.3	8.6	2019	[66]
Malaysia	1,20	20	40	4.7–16.9	—	—	—	2019	[67]
Nigeria	1	70	69	—	—	0–97.0	8.9	2019	[68]
Greece	2,5,6,7,8,9,13,14,15,16,18,19	29	69	LOD–132.7	9.9	—	—	2020	[69]
Indonesia	1	6	50	39.3–139.5	—	—	—	2020	[70]

AFB₁ = Aflatoxin B₁, Total AFs = Total Aflatoxins, n = number of tested samples.

Number of spice category: [1] Red chilli; [2] Aniseed; [3] Black pepper; [4] Caraway; [5] Cinnamon; [6] Coriander; [7] Cumin; [8] Ginger; [9] Red pepper; [10] Clove; [11] Sweet cumin; [12] Sweet pepper; [13] Turmeric; [14] Curry; [15] Paprika; [16] Cardamom; [17] Garlic; [18] Fenugreek; [19] Mix spices.

Table 2.

The worldwide occurrence of AFB₁ and total AFs with respect to each unique spice.

Composite spices are common in South Asian countries (Bangladesh, India, Pakistan and Sri Lanka) due to enrich aroma, color and flavor in variety of cooked meals. Yet no survey is accessible on AFs incidence in composite spices. Some studies have informed that spices are considerably more contaminated with AFs than other foods [71]. Furthermore, chilli, red and black peppers were found to be more contaminated as compare to other spices as reported from India, Pakistan, Turkey, Iran, Bangladesh, Indonesia and Nigeria [4, 72]. The samples of ground chilli were found more exposed to AFs occurrence than whole or uncut red chilli. It is because of the possibility of inferior raw material usage for powder chilli production. Also, the tendency of powder to be hygroscopic makes it susceptible for high AFs contamination [73]. The inappropriate handling, processing and high amount of fat content in black pepper and the solubility of AFs in fat is most likely the reason of high level of AFs contamination. In addition, the presence of essential oils may prevent the occurrence of AFs in crude spices [74].

The antifungal and anti-toxicogenic properties of ginger, turmeric, clove, cinnamon, garlic and cumin can maintain the AFs occurrence at lower level [75, 76]. Some spices show antifungal activity and disrupt the integrity of fungi cell wall which creates AFs [77]. Also, some stated that the extract of turmeric can downregulate the gene expression in the biosynthesis of AFs in *A. flavus* [78]. The lack of AFs in cinnamon and cumin is due to the inhibition of the aflatoxigenic fungi by essential oils and aromatic constituents in these plants. The cinnamon is likely not to be a good substrate for aflatoxigenic fungi development and accumulation. Additionally, the effect of 09 oils studied against the growth and the toxicity of *A. parasiticus* [79]. The clove oil was found capable to inhibit the development of fungi to limit the formation of discontinue the AFs biosynthesis [80].

Considering highly reported contamination with AFs in spices particular chillis and peppers regular monitoring of the imported spices are highly recommended to maintain the food quality. Advance studies are still needed to address the source of occurrence and to control the AFs level in the spice from pre-harvesting to post-harvesting and from packaging, storage and shipment stages. A worldwide potential risk for AFs contamination may occur during prolonged storing of spices in poor temperature and moisture control. The factor of storage environmental conditions plays a major role in the occurrence of secondary metabolites such as AFs.

7. Mitigation of aflatoxins in spices

The widespread elimination or inhibition of AFs contamination during pre- and post-harvest steps is not an easy work however strategies to control fungal growth are essential to minimize the exposure to humans. Numerous methods for the detoxification or elimination of AFs by means of physical, chemical and biological approaches have been proposed [81]. The product safety outcomes of these methods and reducing agents are not clear.

Approaches to address AFs fall under two main areas. The first includes reducing AFs occurrence in the growing cycle by applying good agricultural practices and the other is mitigating the accelerated toxin growth in the post-harvest supply chain both approaches reduce AFs levels in food commodities. Farmers need to use those crop varieties robust to native growing environment, mainly drought, insects and pests, also show resistance to fungal contamination. Postharvest control of humidity is a key to reducing chances of AFs contamination. Irrigation and fungicides can develop plant health to resist the AFs-producing fungus. Solar dryer is also a solution to control moistness in spices earlier to storage.

AFs occurrence could be reduced by different inhibition methods. The toxins levels could be minimized when the defected chilies like midget, dwarf, damaged and broken are physically sorted from achieving an average of 78% reduction in toxin content [82]. The use of gamma radiation can be helpful for the protection of chilies with respect to the production of AFs during storage [83]. In addition, some novel detoxification technologies including a microwave, ultraviolet, electrolyzed water, ozone, pulsed light, cold plasma and gamma irradiation in a support with biological, physical, chemical or genetic engineering methods have the potential to detoxify [84–87]. The application of each technique has its benefits and drawbacks. Consequently, biocontrol processes in synchrony with other physical and chemical methods with improved packaging materials should be implemented to attain spices safety and security.

By the execution of advanced agricultural technologies, good agricultural practices, good manufacturing practices and good storage practices can mitigate the AFs occurrence or contamination [88]. AFs contamination occurs in pockets of high concentration which are not randomly dispersed throughout the commodity [89]. Thus, sampling is of key importance before sample preparation in laboratory. The sampling, sample handling and analyses are not yet standardized at growers and farmers eventually the users are at risk. Therefore, attention must be taken in the determination of laboratory results and quality testing should be performed from ISO-17025 accredited or similar laboratories.

Furthermore, the unpacked composite spices are susceptible to the AFs occurrence because of direct exposure to climate. The higher levels of AFs presence could be credited to tropical condition which may favor the spread of toxigenic fungi [90]. Organization of American States and Mayan Reserve Foundation jointly reported the corrective measures to reduce AFs contamination in chilies such as storage at low relative humidity and temperature, shorten the drying time and quick supplying to the user. And last but not least, the skilled personnel to involve in these processes [91]. Hence, consumers are guided to take measures such as procure from reliable retailers, store food in cool conditions and avoid unpacked products.

8. Conclusion

Aflatoxins contaminated spices are associated with severe risks to the consumers as these spices are part of food particularly in the Asian cooking. It is essential for legislative bodies to monitor AFs occurrence and harmful effects in spices to endorse that toxins are not prevailing at levels that may harmful to consumer health. Also, harvesting, drying, storage and transportation should be cautiously organized to control fungus growth. AFs occurrence can be controlled at pre- and post-harvest positions by applying good agricultural, good manufacturing and good storage practices. Further, the unique innovative processing technologies in combination either with genetic engineering or with physical, chemical or biological approaches have the potential to improve the capability of AFs decontamination as well as to overcome the limitations of any specific technology.

Acknowledgements

The authors are enthusiastically thanks to the Pakistan Council of Scientific & Industrial Research (PCSIR), Karachi Laboratories Complex, Pakistan for the support of Food & Feed Safety research.

Conflict of interest

The authors declare no conflict of interest.

IntechOpen

IntechOpen

Author details

Farman Ahmed* and Muhammad Asif Asghar
Food and Feed Safety Laboratory, Food and Marine Resources Research Centre,
Pakistan Council of Scientific and Industrial Research (PCSIR), Karachi
Laboratories Complex, Karachi, Pakistan

*Address all correspondence to: ahmed.farman@gmail.com

IntechOpen

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Alirezalu K, Pateiro M, Yaghoubi M, Alirezalu A, Peighambardoust SH, Lorenzo JM. Phytochemical constituents, advanced extraction technologies and techno-functional properties of selected Mediterranean plants for use in meat products. A comprehensive review. *Trends in Food Science & Technology*. 2020.
- [2] Pitt JI, Miller JD. A concise history of mycotoxin research. *Journal of agricultural and food chemistry*. 2017;65(33):7021-33.
- [3] Kabak B, Dobson AD. Mycotoxins in spices and herbs—An update. *Critical reviews in food science and nutrition*. 2017;57(1):18-34.
- [4] Pickova D, Ostry V, Malir J, Toman J, Malir F. A Review on Mycotoxins and Microfungi in Spices in the Light of the Last Five Years. *Toxins*. 2020;12(12):789.
- [5] Azzoune N, Mokrane S, Riba A, Bouras N, Verheecke C, Sabaou N, et al. Contamination of common spices by aflatoxigenic fungi and aflatoxin B1 in Algeria. *Quality Assurance and Safety of Crops & Foods*. 2015;8(1):137-144.
- [6] Asghar MA, Zahir E, Ranttila S, Ahmed A, Iqbal J. Aflatoxins in composite spices collected from local markets of Karachi, Pakistan. *Food Additives & Contaminants: Part B*. 2016;9(2):113-119.
- [7] Bennett JW. An overview of the genus *Aspergillus*. *Aspergillus: molecular biology and genomics*. 2010:1-17.
- [8] Arce-López B, Lizarraga E, Vettorazzi A, González-Peñas E. Human biomonitoring of mycotoxins in blood, plasma and serum in recent years: a review. *Toxins*. 2020;12(3):147.
- [9] Asghar MA, Ahmed A, Iqbal J. Aflatoxins and ochratoxin A in export quality raisins collected from different areas of Pakistan. *Food Additives & Contaminants: Part B*. 2016;9(1):51-58.
- [10] Alshannaq A, Yu J-H. Occurrence, toxicity, and analysis of major mycotoxins in food. *International journal of environmental research and public health*. 2017;14(6):632.
- [11] Cancer IAFRo. Monographs on the evaluation of carcinogenic risks to humans. <http://monographsiarcfr/ENG/Classification/index.php>. 2006.
- [12] Shi D, Liao S, Guo S, Li H, Yang M, Tang Z. Protective effects of selenium on aflatoxin B 1-induced mitochondrial permeability transition, DNA damage, and histological alterations in duckling liver. *Biological trace element research*. 2015;163(1):162-168.
- [13] Akhtar S, Riaz M, Naeem I, Gong YY, Ismail A, Hussain M, et al. Risk assessment of aflatoxins and selected heavy metals through intake of branded and non-branded spices collected from the markets of Multan city of Pakistan. *Food Control*. 2020;112:107132.
- [14] Singh P, Cotty PJ. Aflatoxin contamination of dried red chilies: Contrasts between the United States and Nigeria, two markets differing in regulation enforcement. *Food Control*. 2017;80:374-379.
- [15] Do KH, An TJ, Oh S-K, Moon Y. Nation-based occurrence and endogenous biological reduction of mycotoxins in medicinal herbs and spices. *Toxins*. 2015;7(10):4111-4130.
- [16] Cho S-H, Lee C-H, Jang M-R, Son Y-W, Lee S-M, Choi I-S, et al. Aflatoxins contamination in spices and processed

- spice products commercialized in Korea. *Food Chemistry*. 2008;107(3):1283-1288.
- [17] FAOSTAT. Food and Agriculture Organization of the United Nations. Available online: <http://www.fao.org/faostat/en/#data/QC/visualize>. (accessed on 05 February 2021).
- [18] Seasoning and Spices Market - Growth T, COVID-19 Impact, and Forecasts (2021-2026). Available online: <https://www.mordorintelligence.com/industry-reports/seasoning-and-spices-market>. (accessed on 05 February 2021). 2013.
- [19] Dövényi-Nagy T, Rácz C, Molnár K, Bakó K, Szláma Z, Józwiak Á, et al. Pre-Harvest Modelling and Mitigation of Aflatoxins in Maize in a Changing Climatic Environment—A Review. *Toxins*. 2020;12(12):768.
- [20] Olarte RA. Population Dynamics of Intra-and Inter-Specific Crosses and the Effect of Biocontrol on Natural Populations of *Aspergillus* Species: North Carolina State University; 2014.
- [21] Schmidt-Heydt M, Rüfer CE, Abdel-Hadi A, Magan N, Geisen R. The production of aflatoxin B₁ or G₁ by *Aspergillus parasiticus* at various combinations of temperature and water activity is related to the ratio of aflS to aflR expression. *Mycotoxin Research*. 2010;26(4):241-246.
- [22] Cotty PJ, Jaime-Garcia R. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. *International journal of food microbiology*. 2007;119(1-2):109-115.
- [23] Herrera M, Anadón R, Iqbal SZ, Bailly J, Ariño A. Climate change and food safety. *Food Safety*: Springer; 2016. p. 149-160.
- [24] Dohlman E. Mycotoxin hazards and regulations. *International Trade and Food Safety*. 2003;97.
- [25] Food U, Administration D. Guidance for industry: action levels for poisonous or deleterious substances in human food and animal feed. USFDA, Washington, DC. 2000.
- [26] Food, Administration D. Guidance for industry: Action levels for poisonous or deleterious substances in human food and animal feed. 2000. 2010.
- [27] Commission E. Commission Regulation (EC) 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;50:8-12.
- [28] Zhang K, Banerjee K. A Review: Sample Preparation and Chromatographic Technologies for Detection of Aflatoxins in Foods. *Toxins*. 2020;12(9):539.
- [29] Bae SE, Cho SY, Won YD, Lee SH, Park HJ. A comparative study of the different analytical methods for analysis of S-allyl cysteine in black garlic by HPLC. *LWT-Food Science and Technology*. 2012;46(2):532-535.
- [30] O'Riordan MJ, Wilkinson MG. Comparison of analytical methods for aflatoxin determination in commercial chilli spice preparations and subsequent development of an improved method. *Food Control*. 2009;20(8):700-705.
- [31] Khan MA, Asghar MA, Iqbal J, Ahmed A, Shamsuddin ZA. Aflatoxins contamination and prevention in red chillies (*Capsicum annum* L.) in Pakistan. *Food Additives & Contaminants: Part B*. 2014;7(1):1-6.
- [32] Delaunay N, Combès A, Pichon V. Immunoaffinity Extraction and Alternative Approaches for the Analysis of Toxins in Environmental, Food or Biological Matrices. *Toxins*. 2020;12(12):795.

- [33] Sirhan A, Tan G, Wong R. Method validation in the determination of aflatoxins in noodle samples using the QuEChERS method and high performance liquid chromatography coupled to a fluorescence detector (HPLC-FLD). *Food Control*. 2011;22:1839-1843.
- [34] Sirhan AY, Tan GH, Al-Shunnaq A, Abdulra'uf L, Wong RC. QuEChERS-HPLC method for aflatoxin detection of domestic and imported food in Jordan. *Journal of Liquid Chromatography & Related Technologies*. 2014;37(3):321-342.
- [35] Sapsford KE, Taitt CR, Fertig S, Moore MH, Lassman ME, Maragos CM, et al. Indirect competitive immunoassay for detection of aflatoxin B1 in corn and nut products using the array biosensor. *Biosensors and bioelectronics*. 2006;21(12):2298-2305.
- [36] Iqbal J, Asghar MA, Ahmed A, Khan MA, Jamil K. Aflatoxins contamination in Pakistani brown rice: a comparison of TLC, HPLC, LC-MS/MS and ELISA techniques. *Toxicology mechanisms and methods*. 2014;24(8):544-551.
- [37] Turner NW, Subrahmanyam S, Piletsky SA. Analytical methods for determination of mycotoxins: a review. *Analytica chimica acta*. 2009;632(2):168-180.
- [38] Bueno D, Istamboulie G, Muñoz R, Marty JL. Determination of mycotoxins in food: a review of bioanalytical to analytical methods. *Applied Spectroscopy Reviews*. 2015;50(9):728-774.
- [39] Hosseini S, Vázquez-Villegas P, Rito-Palomares M, Martínez-Chapa SO. Advantages, disadvantages and modifications of conventional ELISA. *Enzyme-linked Immunosorbent Assay (ELISA)*: Springer; 2018. p. 67-115.
- [40] Asghar MA, Iqbal J, Ahmed A, Khan MA, Shamsuddin ZA, Jamil K. Development and validation of a high-performance liquid chromatography method with post-column derivatization for the detection of aflatoxins in cereals and grains. *Toxicology and industrial health*. 2016;32(6):1122-1134.
- [41] Espinosa ET, Askar KA, Naccha Torres LR, Olvera RM, Santa Anna JPC. Quantification of aflatoxins in corn distributed in the city of Monterrey, Mexico. *Food Additives & Contaminants*. 1995;12(3):383-386.
- [42] Jansen H, Jansen R, Brinkman UT, Frei R. Fluorescence enhancement for aflatoxins in HPLC by post-column split-flow iodine addition from a solid-phase iodine reservoir. *Chromatographia*. 1987;24(1):555-559.
- [43] Chiavaro E, Dall'Asta C, Galaverna G, Biancardi A, Gambarelli E, Dossena A, et al. New reversed-phase liquid chromatographic method to detect aflatoxins in food and feed with cyclodextrins as fluorescence enhancers added to the eluent. *Journal of Chromatography A*. 2001;937(1-2):31-40.
- [44] Garner RC, Whattam MM, Taylor PJ, Stow MW. Analysis of United Kingdom purchased spices for aflatoxins using an immunoaffinity column clean-up procedure followed by high-performance liquid chromatography. *Journal of Chromatography A*. 1993;648(2):485-490.
- [45] Shepherd MJ, Gilbert J. An investigation of HPLC post-column iodination conditions for the enhancement of aflatoxin B1 fluorescence. *Food Additives & Contaminants*. 1984;1(4):325-335.
- [46] Kok WT. Derivatization reactions for the determination of aflatoxins by liquid chromatography with

fluorescence detection. *Journal of Chromatography B: Biomedical Sciences and Applications*. 1994;659(1-2):127-137.

[47] Shephard GS. Determination of mycotoxins in human foods. *Chemical Society Reviews*. 2008;37(11):2468-2477.

[48] Jeswal P, Kumar D. Mycobiota and natural incidence of aflatoxins, ochratoxin A, and citrinin in Indian spices confirmed by LC-MS/MS. *International journal of microbiology*. 2015;2015.

[49] Herebian D, Zühlke S, Lamshöft M, Spiteller M. Multi-mycotoxin analysis in complex biological matrices using LC-ESI/MS: Experimental study using triple stage quadrupole and LTQ-Orbitrap. *Journal of separation science*. 2009;32(7):939-948.

[50] Tosun H, Arslan R. Determination of aflatoxin B1 levels in organic spices and herbs. *The Scientific World Journal*. 2013;2013.

[51] Akhund S, Akram A, Hanif NQ, Qureshi R, Naz F, Nayyar BG. Pre-harvest aflatoxins and *Aspergillus flavus* contamination in variable germplasms of red chillies from Kunri, Pakistan. *Mycotoxin Research*. 2017;33(2):147-155.

[52] Karaaslan M, Arslanğray Y. Aflatoxins B 1, B 2, G 1, and G 2 contamination in ground red peppers commercialized in Sanliurfa, Turkey. *Environmental monitoring and assessment*. 2015;187(4):1-9.

[53] Golge O, Hepsag F, Kabak B. Incidence and level of aflatoxin contamination in chilli commercialised in Turkey. *Food Control*. 2013;33(2):514-520.

[54] Mozaffari Nejad AS, Sabouri Ghannad M, Kamkar A. Determination of aflatoxin B1 levels in Iranian and

Indian spices by ELISA method. *Toxin Reviews*. 2014;33(4):151-154.

[55] Barani A, Nasiri Z, Jarrah N. Natural occurrence of aflatoxins in commercial pepper in Iran. *Food and Agricultural Immunology*. 2016;27(4):570-576.

[56] Jalili M. Natural occurrence of aflatoxins contamination in commercial spices in Iran. *Iranian Journal of Health, Safety and Environment*. 2016;3(2):513-517.

[57] Motloung L, De Saeger S, De Boevre M, Detavernier C, Audenaert K, Adebo O, et al. Study on mycotoxin contamination in South African food spices. *World Mycotoxin Journal*. 2018;11(3):401-409.

[58] Khazaeli P, Mehrabani M, Heidari MR, Asadikaram G, Najafi ML. Prevalence of aflatoxin contamination in herbs and spices in different regions of Iran. *Iranian journal of public health*. 2017;46(11):1540.

[59] Ali N, Hashim NH, Shuib NS. Natural occurrence of aflatoxins and ochratoxin A in processed spices marketed in Malaysia. *Food Additives & Contaminants: Part A*. 2015;32(4):518-532.

[60] Gherbawy YA, Shebany YM, Hussein MA, Maghraby TA. Molecular detection of mycobiota and aflatoxin contamination of chili. *Archives of Biological Sciences*. 2015;67(1):223-234.

[61] Gambacorta L, Magistà D, Perrone G, Murgolo S, Logrieco A, Solfrizzo M. Co-occurrence of toxigenic moulds, aflatoxins, ochratoxin A, *Fusarium* and *Alternaria* mycotoxins in fresh sweet peppers (*Capsicum annum*) and their processed products. *World Mycotoxin Journal*. 2018;11(1):159-174.

[62] Fundikira SS. Aflatoxin contamination of marketed spices in

Tanzania: a case study of Dar es salaam: Sokoine University of Agriculture; 2018.

[63] Zahra N, Khan M, Mehmood Z, Saeed M, Kalim I, Ahmad I, et al. Determination of aflatoxins in spices and dried fruits. *Journal of Scientific Research*. 2018;10(3):315-321.

[64] El Darra N, Gambacorta L, Solfrizzo M. Multimycotoxins occurrence in spices and herbs commercialized in Lebanon. *Food Control*. 2019;95:63-70.

[65] Hossain MN, Talukder A, Afroze F, Rahim MM, Begum S, Haque MZ, et al. Identification of aflatoxigenic fungi and detection of their aflatoxin in red chilli (*Capsicum annum*) samples using direct cultural method and HPLC. *Advances in Microbiology*. 2018;8(1):42-53.

[66] Zareshahrabadi Z, Bahmyari R, Nouraei H, Khodadadi H, Mehryar P, Asadian F, et al. Detection of Aflatoxin and Ochratoxin A in Spices by High-Performance Liquid Chromatography. *Journal of Food Quality*. 2020;2020.

[67] Alsharif AMA, Choo Y-M, Tan G-H. Detection of five mycotoxins in different food matrices in the Malaysian market by using validated liquid chromatography electrospray ionization triple quadrupole mass spectrometry. *Toxins*. 2019;11(4):196.

[68] Ezekiel CN, Ortega-Beltran A, Oyedeji EO, Atehnkeng J, Kössler P, Tairu F, et al. Aflatoxin in chili peppers in Nigeria: extent of contamination and control using atoxigenic *Aspergillus flavus* genotypes as biocontrol agents. *Toxins*. 2019;11(7):429.

[69] Koutsias I, Kollia E, Makri K, Markaki P, Proestos C. Occurrence and Risk Assessment of Aflatoxin B1 in Spices Marketed in Greece. *Analytical Letters*. 2020:1-14.

[70] Wikandari R, Mayningsih IC, Sari MDP, Purwandari FA, Setyaningsih W, Rahayu ES, et al. Assessment of microbiological quality and mycotoxin in dried chili by morphological identification, molecular detection, and chromatography analysis. *International journal of environmental research and public health*. 2020;17(6):1847.

[71] El-Dawy EGAE, Yassein AS, El-Said AH. Detection of mycobiota, aflatoxigenic and ochratoxigenic genes, and cytotoxic ability in spices. *Food science & nutrition*. 2019;7(8):2595-2604.

[72] Jacxsens L, De Meulenaer B. Risk assessment of mycotoxins and predictive mycology in Sri Lankan spices: Chilli and pepper. *Procedia food science*. 2016;6:326-330.

[73] Iqbal SZ, Paterson RRM, Bhatti IA, Asi MR. Comparing aflatoxin contamination in chilies from Punjab, Pakistan produced in summer and winter. *Mycotoxin Research*. 2011;27(2):75-80.

[74] Hammami W, Fiori S, Al Thani R, Kali NA, Balmas V, Migheli Q, et al. Fungal and aflatoxin contamination of marketed spices. *Food Control*. 2014;37:177-181.

[75] Kaefer CM, Milner JA. Herbs and spices in cancer prevention and treatment. *Herbal Medicine: Biomolecular and Clinical Aspects* 2nd edition. 2011.

[76] Císarová M, Hleba L, Medo J, Tančinová D, Mašková Z, Čuboň J, et al. The in vitro and in situ effect of selected essential oils in vapour phase against bread spoilage toxicogenic aspergilli. *Food Control*. 2020;110:107007.

[77] Liu Q, Meng X, Li Y, Zhao C-N, Tang G-Y, Li H-B. Antibacterial and antifungal activities of spices.

International journal of molecular sciences. 2017;18(6):1283.

[78] Mohajeri M, Behnam B, Cicero AF, Sahebkar A. Protective effects of curcumin against aflatoxicosis: A comprehensive review. *Journal of cellular physiology*. 2018;233(4):3552-3577.

[79] Juglal S, Govinden R, Odhav B. Spice oils for the control of co-occurring mycotoxin-producing fungi. *Journal of food protection*. 2002;65(4):683-687.

[80] Combrinck S, Regnier T, Kamatou GP. In vitro activity of eighteen essential oils and some major components against common postharvest fungal pathogens of fruit. *Industrial Crops and Products*. 2011;33(2):344-349.

[81] Jalili M. A review on aflatoxins reduction in food. *Iranian Journal of Health, Safety and Environment*. 2016;3(1):445-459.

[82] Khan M, Asghar M, Ahmed A, Iqbal J, Shamsuddin Z. Reduction of aflatoxins in dundi-cut whole red chillies (*Capsicum indicum*) by manual sorting technique. *Science Technology and Development*. 2013;32(1):16-23.

[83] Iqbal SZ, Bhatti IA, Asi MR, Zuber M, Shahid M, Parveen I. Effect of γ irradiation on fungal load and aflatoxins reduction in red chillies. *Radiation Physics and Chemistry*. 2013;82:80-84.

[84] Pankaj S, Shi H, Keener KM. A review of novel physical and chemical decontamination technologies for aflatoxin in food. *Trends in Food Science & Technology*. 2018;71:73-83.

[85] Guo Y, Zhao L, Ma Q, Ji C. Novel strategies for degradation of aflatoxins in food and feed: A review. *Food Research International*. 2020:109878.

[86] Tripathi S, Mishra H. Enzymatic coupled with UV degradation of aflatoxin B1 in red chili powder. *Journal of Food Quality*. 2010;33:186-203.

[87] Kamber U, Gülbaz G, Aksu P, Doğan A. Detoxification of aflatoxin B1 in red pepper (*Capsicum annuum* L.) by ozone treatment and its effect on microbiological and sensory quality. *Journal of Food Processing and Preservation*. 2017;41(5):e13102.

[88] Watson I, Kamble P, Shanks C, Khan Z, El Darra N. Decontamination of chilli flakes in a fluidized bed using combined technologies: Infrared, UV and ozone. *Innovative Food Science & Emerging Technologies*. 2020;59:102248.

[89] George W, Latimer J. Official method of analysis of AOAC. AOAC International. 2019.

[90] Afsah-Hejri L, Jinap S, Hajeb P, Radu S, Shakibazadeh S. A review on mycotoxins in food and feed: Malaysia case study. *Comprehensive Reviews in Food Science and Food Safety*. 2013;12(6):629-651.

[91] Sinha A, Petersen J. Caribbean hot pepper production and post harvest manual. FAO/Caribbean Agricultural Research and Development Institute, Rome, Italy. 2011.