Annex E – Pre- and post-harvest strategies to mitigate mycotoxin contamination in spices

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1. The crops

* 1. Spices

Spices are consumed worldwide and their use is markedly influenced by each region’s traditional consumption habits. The increased globalization favours cultural exchange, in which food is included, introducing new options to the various common dishes in the different countries. The use of different spices varieties is nowadays spread all over the world. Because of spices plants’ environmental needs for growth, these are prone to be affected by fungi, and, consequently, to mycotoxin contamination since pre-harvest stages. All the subsequent steps until the achievement of the final product can increase or decrease contamination levels, which requires an integrated vision to effectively control their safety.

Spices are way too diverse, making it difficult to completely assess contamination risks. Therefore, selecting some major spices species according to their consumption rates and safety risks is the best way to evaluate the actual situation on this matter. According to the limits established in the European Union, to the notifications on the Rapid Alert System for Food and Feed (RASFF) portal and to published scientific papers, peppers (*Capsicum* spp.), peppercorns (or black or white pepper - *Piper* spp.), nutmeg (*Myristica* *fragrans*), ginger (*Zingiber* *officinale*) and turmeric (*Curcuma* *longa*) have been considered to be the key spices regarding exposure risk. These species can also be included as mixtures of spices, which makes them even more difficult to evaluate. The European Commission’s legislation establishes limits for aflatoxins (AFs) and ochratoxin A (OTA), but ongoing and future studies will evaluate the possible need for new legal restrictions. For the above-mentioned spices, the legal limits are of 5 µg/kg for aflatoxin B1 (AFB1) and of 10 µg/kg for total AFs. In case of OTA, distinction is made between the different spices and there is a limit of 20 µg/kg for *Capsicum* spp. products and of 15 µg/kg for *Piper* spp., *Myristica* *fragrans*, *Zingiber* *officinale*, *Curcuma* *longa* and mixtures of spices with at least one of the regulated matrixes (European Commission, 2006) (Table 1).

The commerce of spices between different countries has an increased monitoring difficulty, because of the higher uncertainty in practices during the productive chain in the origin country, coupled with the widespread traditional cropping and processing methods, which may not be ideal when dealing with fungal contamination. As an example, the Korean way of drying spices in the sun, particularly red paprika, is the way of ensuring the desired quality, even knowing that the required time and the dependency of environmental parameters can possess threats to the safety of the dried product (Ahn et al., 2010). Keeping these practices requires a stricter control, which is not always present.

Spices can be used in different forms, *e.g.* powders, crushed or whole. These variations can affect fungal contamination at post-harvest stages because of differences in fungal proliferation and mycotoxin production due to the matrix (see Processing section). As reported by Khan, Asghar, Iqbal, Ahmed, and Shamsuddin (2014), it is known that the quality of raw products may differ according to the desired final product. Powders and crushed chillies can be made using lower quality chillies (e.g., broken), which may contribute to the higher AFs levels found in these products, compared to the values in whole chillies (Khan et al., 2014). In India, it is a common practice to grade chillies according to their contamination levels, and even when fungal growth is already visible they are sold, but at lower prices, enabling a widespread consumption of these products by the poorest part of the population, and increasing their AFs exposure (Reddy, Mayi, Reddy, Thirumala-Devi, & Reddy, 2001). This fact, together with the practice of sprinkling water in chilli pods when selling, were shown to increase the risk of AFs exposure, particularly in some population groups that cannot opt for higher quality products (Reddy et al., 2001). This marketing strategy is also applied in Sri Lanka to increase the weight of the product and, consequently, selling incomes (Yogendrarajah, Jacxsens, De Meulenaer, & De Saeger, 2014).

1. Maximum levels of mycotoxins in spices (according to European Commission, 2006)

| Spices product | Maximum levels (μg/kg) | |
| --- | --- | --- |
| Following species of spices:  - *Capsicum* spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne and paprika)  - *Piper* spp. (fruits thereof, including white and black pepper)  - *Myristica fragrans* (nutmeg)  - *Zingiber officinale* (ginger)  - *Curcuma longa* (turmeric)  - Mixtures of spices containing one or more of the above mentioned spices | Aflatoxin B1 | 5 |
| Total aflatoxins | 10 |
| Spices, including dried spices, of:  - *Piper* spp. (fruits thereof, including white and black pepper)  - *Myristica fragrans* (nutmeg)  - *Zingiber officinale* (ginger)  - *Curcuma longa* (turmeric) | Ochratoxin A | 15 |
| Spices, including dried spices, of:  - *Capsicum* spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne and paprika) | Ochratoxin A | 20 |
| Following species of spices:  - Mixtures of spices containing one of the above mentioned spices | Ochratoxin A | 15 |

* + 1. Main fungi in spices

Spices can be exposed to fungal attack and mycotoxin production since pre-harvest stages, which can be a result of contaminated residues present in the soil from previous cultivations (Ahn et al., 2010). Even when mycotoxins are not produced in field, if the level of fungal contamination is high and environmental conditions are favourable, mycotoxin production can occur after harvest and, since their posterior elimination is not that easy, safety can be compromised (Ahn et al., 2010). Table 2 presents summarized data on the potential for mycotoxin production of the main mycotoxigenic genera isolated from spices.

In order to consider mycotoxins’ risks while cropping spices, factors like the environmental conditions, as well as irrigation and fertilization practices need to be taken into account. Seeds selection and the use of resistant varieties can allow a proper protection, while rising production rates and quality to increase farmers’ income (Costa et al., 2019; Khan et al., 2014). The intensity of cultivation is also relevant, since the associated fungal load can be increased as a result of the adopted practices. Particularly, the use of consecutive monocultures can negatively affect microorganisms in soil, favouring the prevalence of phytopathogenic fungi and decreasing antagonistic populations (Ahn et al., 2010; Li et al., 2016). A study showed that consecutive cropping of *Piper* *nigrum* L. affected the microbiome, increasing prevalence of *Aspergillus* and *Fusarium* species. Nevertheless, these were influenced in different ways whether the analysed soil was rhizosphere or non-rhizosphere soil, and reductions in *Pseudomonas* and *Bacillus* genera were also observed, negatively impacting the growth and heath of black pepper, since these are beneficial by competing with other species (Li et al., 2016).

1. Potential as mycotoxin producers of the main fungi isolated from spices

| Spices product | *Aspergillus* | *Penicillium* | *Fusarium* | *Alternaria* |
| --- | --- | --- | --- | --- |
| *Capsicum* spp. | AFs, OTA | \_\_ | DON, FB, T2, ZEN | AOH, AME, TEN |
| *Piper* spp. | AFs, OTA, STER | OTA, CIT | FB | TEN |
| *Myristica* *fragrans* (nutmeg) | AFs, OTA | \_\_ | FB | \_\_ |
| *Zingiber* *officinale* (ginger) | AFs, OTA | \_\_ | \_\_ | \_\_ |
| *Curcuma* *longa* (turmeric) | AFs, OTA | \_\_ | FB | \_\_ |

When harvesting peppers, over-ripening must be avoided since fungal development is favoured in the subsequent phases (Ahn et al., 2010). In fact, after contact with the plant, fungal proliferation to the interior parts causes a rapid deterioration and this is facilitated by damages on the surface, which is more susceptible with increased maturation (Costa et al., 2019). In chillies, once the interior is reached by fungi, the inner alveolar structure can favour spreading (Manda, Adanou, Ardjouma, Adepo, & Dano, 2016). However, even when there is no apparent fungal growth, mycotoxins can be produced and spread into the inner parts. In fact, Yang et al. (2016) studied *Aspergillus* *flavus* inoculated ginger and showed that AFB1 was greatly present, and aflatoxin B2 (AFB2) in reduced amounts, in the interior parts after an inoculation in the surface and even with no visual growth of the fungus. The same experiment with *Aspergillus* *carbonarius* and OTA could not led to conclusions since the levels of the toxin were always below the quantification limit (Yang et al., 2016).

Spices, like other plants, have defence mechanisms, producing compounds likely to inhibit microbial attack, which makes them appealing to be applied in other fields (Seo et al., 2014). This presence needs to be considered when testing for contaminations, since microorganisms can be inhibited during the experiment and mislead the analysts. Klebukowska, Zadernowska, and Chajecka-Wierzchowska (2015) described this, recommending the application of higher dilutions in the assays so that the antimicrobial compounds can be diluted, allowing growth in case of contamination. Data on the use of essential oils or some extracts from different spices to inhibit microbial growth are diverse (Muhammad et al., 2016; Mwangi, Nguta, & Muriuki, 2014).

Particularly in *Capsicum* spp., the search for compounds with antimicrobial properties is a common research topic, along with the potential role of mycotoxins in the interaction between fungi and plant. Santos, Kasper, Sardinas, et al. (2010) and Santos, Kasper, Gil-Serna, et al. (2010) have shown the efficacy of the carotenoids capsanthin and capsorubin against *Aspergillus* species, and AFs and OTA production. In addition, *Capsicum* samples with higher capsaicin levels, which is responsible for the pungency, were associated to lower mycotoxin levels (Santos, Marín, Sanchis, & Ramos, 2010). Also, Tewksbury et al. (2008) found that seeds from pungent chillies presented less *Fusarium* deterioration and hypothesised that increasing populations of pungent plants can be a result of natural selection to protect the plants from microbial spoilage. A previous study, contrarily, showed a weak inhibition of *A*. *flavus* growth and AFs production by capsaicin, compared to the effective results obtained with capsanthin (Masood, Dogra, & Jha, 1994). In fact, both in hot and sweet *Capsicum*, the exact interactions occurring between these compounds and fungal development are not yet fully understood during plant development, specifically, in which conditions inhibition or even stimulation can occur, and there are still progresses to be made in this field (Costa et al., 2019).

In field, the usually associated fungal genera in *Capsicum* plants are *Alternaria*, *Cladosporium*, *Fusarium* and *Rhizopus* (Costa et al., 2019). Other fungi may be present and in some cases follow the entire chain until more conductive ecological conditions allow their proliferation, as it can be the case of *Aspergillus* and *Penicillium* species.

Santos et al. (2011) reported different *Capsicum* powder samples, namely paprika, chilli and smoked paprika, to have *Wallemia* sp., *Ulocladium* sp., *Rhizopus* spp., *Penicillium* spp., *Mucor* spp., *Fusarium* spp., *Eurotium* spp., *Cladosporium* spp., *Aspergillus* spp. and *Alternaria* spp. In samples of paprika, smoked paprika and chilli, it was observed that the prevalent contamination was by the *Aspergillus* section *Nigri*, particularly by *Aspergillus* *niger* aggregate (Santos et al., 2011).

A study with chilli and black and white peppers collected in Sri Lanka found *A*. *flavus*, *A.* *parasiticus*, *A*. *niger* and *Penicillium* spp. to be the predominant fungi (Jacxsens, Pratheeb, Yogendrarajaha, & Meulenaer, 2016). It was observed that contamination with moulds was significantly higher in black pepper samples, compared to white pepper ones (Jacxsens et al., 2016). This can indicate a protective effect of the exterior part of the peppercorn that make it harder for fungi to spread in white pepper products.

A variety of spices sold in India was also analysed for fungal mycoflora, and in red chilli, black pepper, turmeric and dry ginger samples, *Aspergillus*, *Penicillium* and *Fusarium* species were present (Jeswal & Kumar, 2015). In red chili and black pepper, species from *Rhizopus* and *Mucor* *hiemalis* have also been detected, while *Alternaria* *alternata* and *Chaetomium* *globosum* were only detected in red chili (Jeswal & Kumar, 2015).

In ginger, *Penicillium*, *Fusarium*, and *Mortierella* genera are known contaminants (J. Liu et al., 2014; Overy & Frisvad, 2005; Stirling, 2004). Particularly, *Penicillium* *brevicompactum* and *Penicillium* *crustosum* (Overy & Frisvad, 2005), and *Fusarium* *oxysporum* f. sp. *zingiberi* were determined to be the most frequently isolates in spoiled ginger samples (Stirling, 2004). In nutmeg kernels, Nurtjahja, Dharmaputra, Rahayu, and Nazli (2017) found the fungal genera *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, *Penicillium* and *Syncephalastrum* in samples that were to be stored.

As the final commercialized spices are very often mixtures of not only different spices, but other flavouring products, assessing overall risks needs to include these. So, Dutkiewicz et al. (2001) studied the microflora present in air samples of two different Polish facilities of herbs that can be used to produce spices mixtures, namely marjoram herb (*Majorana* *hortensis* Moench), yarrow herb (*Achillea* *millefolium* L.), caraway (*Carum* *carvi* L.), mint (*Mentha* *pulegium* L.), sage (*Salvia* *officinalis* L.), stinging nettle (*Urtica* *dioica* L.), celandine roots (*Chelidonium* *maius* L.), white warty birch (*Betula* *verrucosa* Erh.), peppermint (*Mentha* *piperita* L.), calamus (*Acorus* *calamus* L.) and St. John’s wort (*Hypericum* *perforatum* L.). The *Aspergillus* species were the most frequently found, and other isolated fungi included *A*. *alternata*, *Alternaria* *malvae*, *Alternaria* spp., *Candida* spp., *Fusidium* *terricola*, *Macrosporium* *commune*, *Mucor* spp., *Penicillium* *citrinum*, *Penicillium* spp., *Prophytroma* *tubularis*, *Rhizopus* *nigricans*, *Scopulariopsis* spp., *Trichoderma* *sympodianum* and *Trichoderma* *viride* (Dutkiewicz et al., 2001).

Ecological conditions

The differences in the ecological needs of the contaminant fungi are essential when assessing contamination risks. By knowing the environmental conditions to which the spices where subjected to, a prediction on the level and type of fungal attack can be made and appropriate actions can be taken. A comparative study of *Capsicum* powder samples from Korea and China, found that Chinese samples had lower OTA levels, which was due to drier environmental conditions in this country (Ahn et al., 2010). This knowledge will be particularly relevant because of the predicted climate change that will lead to shifts in the current panorama in all food products, and contaminations with different genera can be expected in regions where they are not yet a concern (Farkas, Beczner, & Mohacsi-Farkas, 2011).

A wide range of studies exists on conductive ecological conditions to fungal growth and mycotoxin production. For *Aspergillus* *westerdijkiae* and *Aspergillus* *steynii*, the optimum growth in paprika-based medium was observed at 28 °C and 0.964 water activity (aw), with the latter presenting a relative lower growth (Gil-Serna, Patino, Cortes, Gonzalez-Jaen, & Vazquez, 2015). When assessing OTA production, it was higher in *A*. *steynii*, with an optimum at 28 °C and 0.995 aw, while the optimum for *A*. *westerdijkiae* was at 24 °C and 0.964 aw (Gil-Serna et al., 2015).

Experiments in red chilli extract agar at 25 °C have shown that *A*. *flavus* strains had a higher growth (including growth rates and lag phases measures) at an aw of 0.99 (Marin, Colom, Sanchis, & Ramos, 2009). By using peppercorns *in* *vitro*, and after analysing growth and lag phases, Pratheeba et al. (2016) created a multi-factorial cardinal model for *A*. *flavus* and *A*. *parasiticus* and stated that optimum conditions to growth were between 30 – 33 °C and 0.87 – 0.92 aw. Simultaneously, the production of sterigmatocystin (STER), AFB1 and the aflatoxins G1 and G2 by *A*. *parasiticus* and just of STER by *A*. *flavus* was observed (Pratheeba et al., 2016).

* + 1. Mycotoxin occurrence

As stated before, spices are way too diverse, making it difficult to completely assess contamination risks. For the purpose of this report, the key spices cultivars for exposure risk to be considered are peppers (*Capsicum* spp.), peppercorns (or black or white pepper - *Piper* spp.), nutmeg (*Myristica* *fragrans*), ginger (*Zingiber* *officinale*) and turmeric (*Curcuma* *longa*). The occurrence of mycotoxins in spices is not very well documented, but the available information makes it possible to conclude on the main mycotoxins occurring and on the co-occurrence.

As with many other commodities, AFs are the main mycotoxins studied with about 60% of the occurrence data published since 2001. The second most reported mycotoxin is OTA (21%). The fact that both these set of mycotoxins represent about 80% of reported data may be attributed to the fact that they are the ones with EU regulation. Besides the above mentioned toxins, *Alternaria* and *Fusarium* mycotoxins have occasionally been reported. Also, most of the reports are focused on a single mycotoxin or a set of closely related ones (e.g., AFs). The co-occurrence of mycotoxins in spices is poorly described, and Table 3 summarise the main ones.

1. Co-occurrence of mycotoxins in spices

| **Mycotoxin** | **Commodity** | **Observation** | **Reference** |
| --- | --- | --- | --- |
| AF; OTA | Red paprika | - Co-occurrence in 67% of samples (21 samples) | Hierro et al., 2008 |
| spices | - Co-occurrence in 2 of 11 samples (one chilli and one paprika) | Tančinová et al., 2014 |
| Red pepper | - Strong evidence of co-occurrence | Fazekas et al, 2014 |
| AF; OTA; ZEN; DON; T2 | Paprika, smoked paprika and chilli | - co-occurrence of AF and OTA in all smoked paprika samples  - co-occurrence of at least two mycotoxins in 82% of paprika and 55% of chilli samples  - few of the paprika samples were simultaneously contaminated with AF, OTA, ZEA and DON  - few of the chilli samples were simultaneously contaminated with AF, OTA, ZEA, DON and T2 | Santos et al, 2011 |

* + 1. Storage management

After harvest, control strategies are mandatory, in order to continue to protect products from fungal development. Washing, drying and sorting can be necessary and these practices should be applied as soon as possible to avoid conditions that lead to fungal growth and mycotoxin production (Manda et al., 2016).

If subsequent processing steps take place, relative humidity (RH) must be controlled during storage, since dried samples can suffer rehydration at this phase. In addition, if fungal populations are mycotoxin-producers, these conductive conditions can lead to this biosynthesis and compromise the safety of the productive chain. In a Spanish study, during the traditional smoked paprika production, the synthesis of penicillic acid and AFs by isolates of *Penicillium* and *Aspergillus* species previously isolated from dried peppers was detected, at a RH ranging from 91 to 97 % (Casquete et al., 2017).

A study in Benin (Akpo-Djenontin, Gbaguidi, Soumanou, & Anihouvi, 2018) evaluated the presence of fungi and AFs in spices and aromatic herb powders collected in markets, supermarkets and at processing plants. When analysed, market samples had the highest total fungal counts and the highest levels of the sum of AFs. The effect of storage time was also evaluated, and samples with more than 3 months had higher total fungal counts than the ones with a shorter storage time. However, the influence of this parameter was not as clear in the samples from supermarkets and processing sites, compared to those collected at markets (Akpo-Djenontin et al., 2018). Interestingly, in this study with different mixtures, containing, among others, garlic, ginger, clove, nutmeg, chilli, cumin, curry and pepper, a relationship was not found between the aw of the final products and both fungal counts and AFs levels. However, the latter finding could be attributed to the low aw (below 0.55) reported for the samples (meaning that storage was performed under controlled and safe conditions).

During storage, more specific techniques can be applied to avoid deterioration caused by fungi. Glowacz and Rees (2016) studied the efficacy of **ozone treatment** in chilli peppers and found that, after 14 days of storage, doses of 0.45 and 0.9 µmol/mol had a more significant effect in decreasing microbial spoilage, than 2 µmol/mol (probably as a result of damages in the skin). Also, considering other quality parameters like colour bleaching and antioxidant activity, 0.9 µmol/mol was the best dose to preserve chillies, with minor negative effects detected in red chilli peppers, when comparing with the green ones (Glowacz & Rees, 2016).

With regards to naturally-based compounds, **essential oils** can also be an alternative in postharvest phases to avoid fungal deterioration. Cinnamon (*Cinnamomum* *zeylanicum*) and *Zanthoxylum* *alatum* Roxb. essential oils have shown promising results in baby ginger and *Piper* *nigrum* L. fruits, respectively (Prakash, Singh, Mishra, & Dubey, 2012; J. Liu et al., 2014). Alternatively, a study with chitosan and oligochitosan also found reductions of deterioration rates, in this case caused by *F*. *oxysporum* in *Zingiber* *officinale* harvested samples, by individually applying each of the compounds at both 1 and 5 g/L, in which the higher dose caused a higher reduction (Y. Liu et al., 2016). Simultaneously, at 5 g/L the enzyme activity of ginger was induced and the analysed quality parameters indicate favourable effects by these compounds (Y. Liu et al., 2016). Based on these results, it was hypothesized that the efficacy of chitosan and its derivative is likely to rely on the activation of defence mechanisms in ginger.

* + 1. Processing and decontamination

Processing steps can influence fungal contamination and mycotoxin levels. In Sri Lankan chilli samples, powders and flakes were found to have higher mycotoxin co-occurrence frequency when compared to whole pods (Yogendrarajah et al., 2014). This fact was also observed in Khan et al. (2014), with higher levels of AFs in powdered and crushed chillies. These findings can be explained by the use of low quality products in processing or by the high hygroscopicity of these products when compared to the whole ones, which enables a higher absorption of air moisture, favouring contamination (Yogendrarajah et al., 2014).

The effect of the particular **matrix characteristics** is also relevant. In pepper powder, the high NaCl concentrations can inhibit some fungal genera because of the low aw, and this can be an advantage to *Aspergillus* and *Penicillium* species, with the latter being prevalent at lower temperatures (Costa et al., 2019).

In some regions, condiments can be obtained by fermenting raw materials. In some cases, advantage can be taken from **fungal activity**, as it is when *A*. *niger* strains are applied to perform the solid-state fermentation of peppercorns to obtain good quality white pepper (Hu, Zhang, Xu, Li, & Liu, 2017). In this case, the dominance of this fungus and the association with some bacterial populations is beneficial to achieve an optimum enzyme activity that ensures a proper peeling (Hu et al., 2017). Besides preserving the products, **fermentation** also influences contamination levels. Chilaka, De Boevre, Atanda, and De Saeger (2018) evaluated the effect of boiling, hull extraction and fermentation steps of three spices traditionally produced in Nigeria from the locally named “African locust bean”, “African castor bean” (which does not include hulls extraction after boiling) and “African mesquite bean” to obtain dawadawa, ogiri, and okpehe. Despite being specific products, the results can influence the application of other processing approaches, and even for different spices. It was observed that, for the studied mycotoxins - deoxynivalenol (DON), fumonisin B1 (FB1), T-2 toxin and zearalenone (ZEN) -, processing of the first spice reduced the toxins from 85 % (ZEN) to 98 % (DON and FB1), the second from 57 % (ZEN) to 81 % (DON), and the last one from 86 % (ZEN) to 100 % (DON) (Chilaka et al., 2018). The differences on mycotoxins reduction can be associated to the different times of fermentation and the inclusion of hull removal.

Changes in processing steps can be explored considering mycotoxins’ risks, so that the adopted conditions lead not only to the required final product, but to a safer consumption. In this line, specific operations can be included. Farawahida, Jinap, Nor-Khaizura, and Samsudin (2017) evaluated the effect of including oil-less **frying** of the used chili powder, **retort processing** of the final product, and both combined, in the peanut sauce processing chain, and concluded that AFs and counts of *A*. *flavus* and *A*. *parasiticus* were reduced by the introduced methods. The greatest result was observed when both oil-less frying of chilli powder and retort processing of the final product were applied, with a final reduction of total AFs of 57 % (Farawahida et al., 2017).

Specific decontamination techniques that can be included in processing are diverse, but the use of **radiation** is usually exploited as postharvest treatment to overcome deterioration problems. Ahn et al. (2010) confirmed the sterilization ability of ultraviolet radiation (UV), with *Aspergillus* *ochraceus* *in* *vitro*. Results from other study showed UV-C efficacy in reducing deterioration and microbial counts in green bell pepper, although raising some issues on the different effects of the applied doses in the inner and outer parts (Rodoni, Concellón, Chaves, & Vicente, 2012). Particularly for moulds, after 12 days, control and treated peppers presented similar counts, possibly indicating that inhibition of visual deterioration could be related to the activation of defence mechanisms that are not directly related to fungal attacks (Rodoni et al., 2012).

By applying different doses of gamma radiation (2, 4 and 6 kGy), Iqbal, Amjad, Asi, and Arino (2012) and Abrar, Anjum, Zahoor, and Nawaz (2009) did not found significant variations in the moisture content of hot and red pepper samples. The effect on mould contamination showed that the highest dose reduced the counts to untraceable values, and these were kept during a storage of 3 months, while for the lowest dose the control of moulds was not achieved. *Aspergillus* species were found to be more resistant than other fungi, and their counts increased significantly more with time, during the 3 months, which could be a result of the elimination of the competing populations (Iqbal et al., 2012). At the same time, no significant reductions were obtained in the AFs levels, and this was attributed to the fact that irradiation effectiveness is higher in larger molecules - as DNA, compared to smaller molecules; so microorganisms are more susceptible than mycotoxins (Calado, Venâncio and Abrunhosa 2014; Iqbal et al., 2012). This conclusion is corroborated by a previous study, in which reduction of AFs by the same irradiation doses was also small (Abrar et al., 2009).

The effect of **microwave cold plasma** treatments has already been studied in red pepper powder and flakes (Farawahida et al., 2017; Kim, Lee, & Min, 2014). *A*. *flavus* was inhibited in the powder when submitted to microwave-powdered plasma at 900 W for 20 minutes at a constant pressure of 667 Pa, by 2.5 ± 0.3, 2.0 ± 0.3, 0.4 ± 0.1, and 0.3 ± 0.1 log spores per g, with N2, He, N2-O2 mixture and He-O2 mixture, respectively. The treatment was proven to cause a reduction on aw levels and its efficacy increased with applied power and time (Kim et al., 2014). In red pepper flakes, a treatment with microwave-combined cold plasma, using the same power and application time, was proven successful in reducing *A*. *flavus* and *Bacillus* *cereus* contaminations, with the highest power densities causing the greatest reductions (Farawahida et al., 2017). In both cases, colour and antioxidant activity were also analysed and no substantial effects were detected, which indicates that this can be an alternative when storing these products (Farawahida et al., 2017; Kim et al., 2014).

Feroz et al. (2016) studied the effect of **heat treatment** at 60 °C on naturally contaminated samples of different types of food, while also applying **low-pressure plasma** on seven dried food samples among those. The heat treatment was applied to 23 different spices, which after analysis, were deemed to be the least susceptible type of food - after 2 hours of treatment, the highest reduction of fungal growth in the studied spices was in turmeric (3.55 log) and the lowest in coriander powder (of 0.27 log). When applying the plasma treatment, after 40 minutes, ginger had the highest reduction among spices (5.91 log), and cumin powder the lowest (2.15 log) (Feroz et al., 2016).

A study with chilli and nutmeg samples imported and packaged in Italy provided data on the effect of a steam treatment at 100 °C in moulds and AFs levels, showing that the treated samples had significantly less mould infection and that nutmeg contaminations (which had higher counts) had an increased reduction using this process (Pesavento et al., 2016). Contrarily, the levels of AFs were higher in the heat-treated samples, possibly indicating a response of the surviving fungi to the stressing conditions (Pesavento et al., 2016).

**Packaging** is essential to achieve a safe storage and the products must be rapidly dried until safe moisture levels are reached. For red chilli, a moisture content under 10 % (w/w) can provide protection from fungal contamination; however, it has to be complemented with the choice of a suitable packaging material, which is essential to avoid further moisture increases, since chilli can absorb water from air (Yogendrarajah et al., 2014). If an effective drying takes place, polyethylene bags can successfully protect products from fungal development and mycotoxin production; otherwise, this material can promote spoilage due to the maintenance of the initial moisture content, which enables fungal growth. Iqbal, Amjad, Asi, and Arino (2011) have studied packaging aspects, by storing dried hot peppers in polyethylene and jute bags, reporting in the first ones a trend towards lower mould counts, including *Aspergillus* species, and AFs levels. They have associated this to water absorption and aeration properties of jute bags. By also evaluating the effect of storage time and temperature, it was found that both the AFs levels and fungal contamination increased, not only with temperatures higher than 20 °C (25 or 30 °C), but also with time (Iqbal et al., 2011).

Abbreviations

|  |  |
| --- | --- |
| AFB1 | Aflatoxin B1 |
| AFB2 | Aflatoxin B2 |
| AFs | Aflatoxins |
| aw | Water activity |
| DON | Deoxynivalenol |
| FB1 | Fumonisin B1 |
| OTA | Ochratoxin A |
| RASFF | Rapid Alert System for Food and Feed |
| RH | Relative humidity |
| STER | Sterigmatocystin |
| T2 | T2 toxin |
| TEN | Tentotoxin |
| UV | Ultraviolet |
| ZEN | Zearalenone |

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