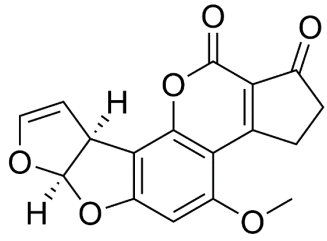




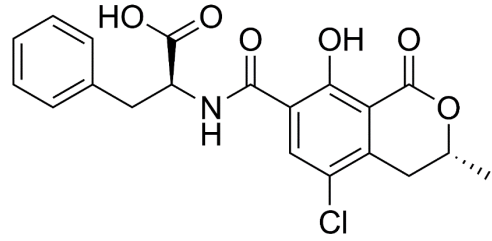
# Mitigating Mycotoxins in Spices

J. David Miller PhD FAIHA  
Distinguished Research Professor

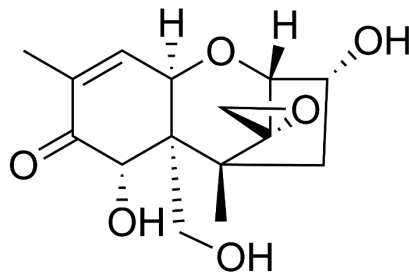




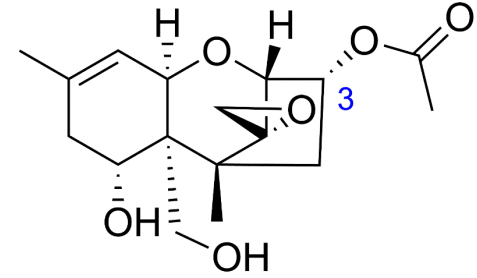
Aflatoxin B1



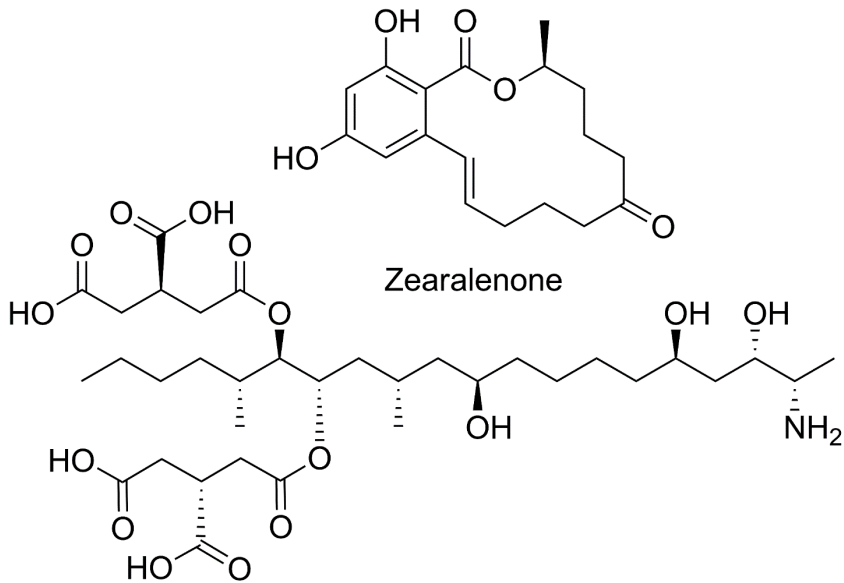
Ochratoxin A



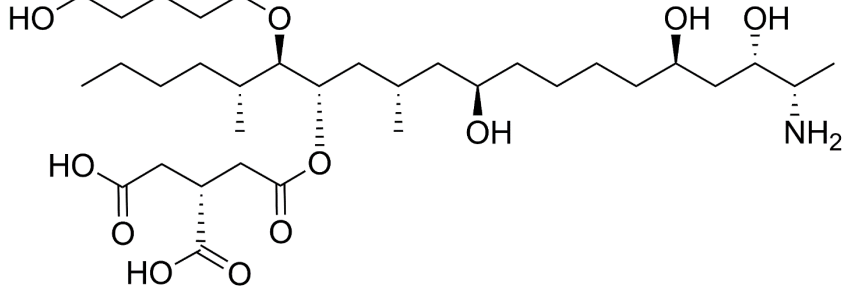
DON



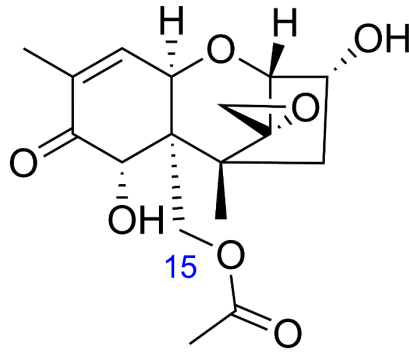
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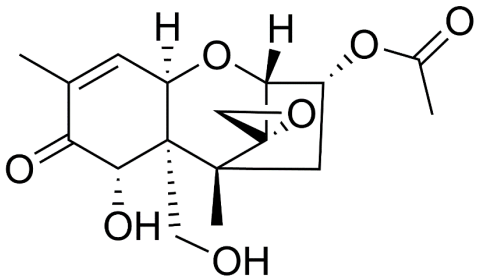
Zearalenone



Fumonisin B1



15ADON



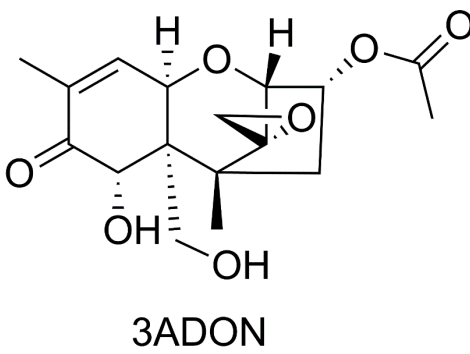
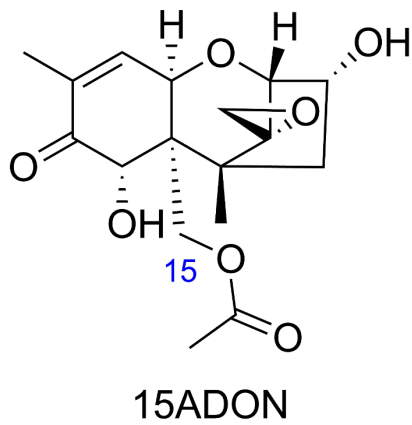
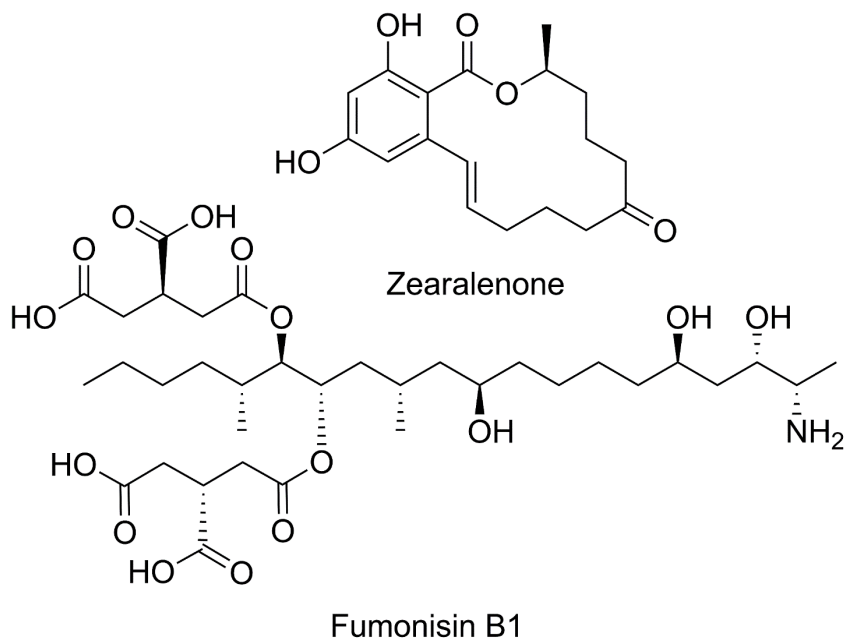
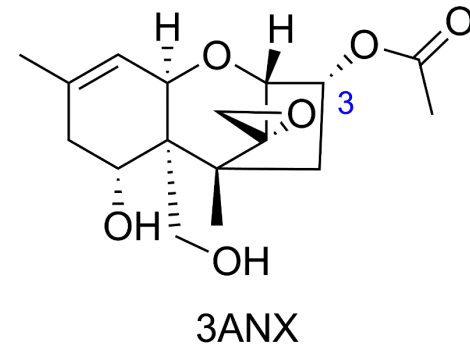
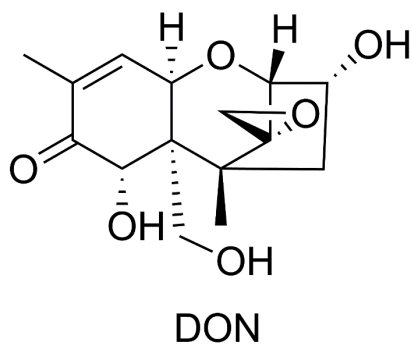
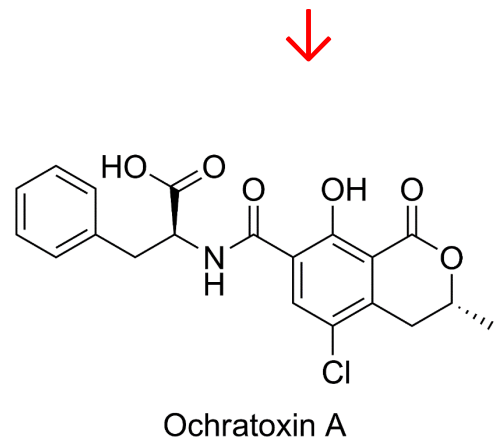
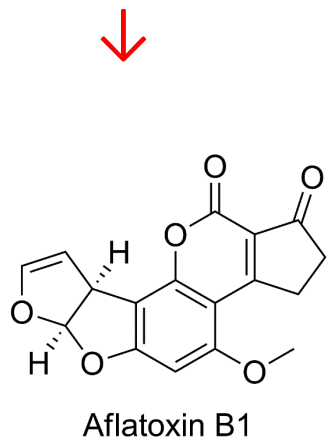
3ADON





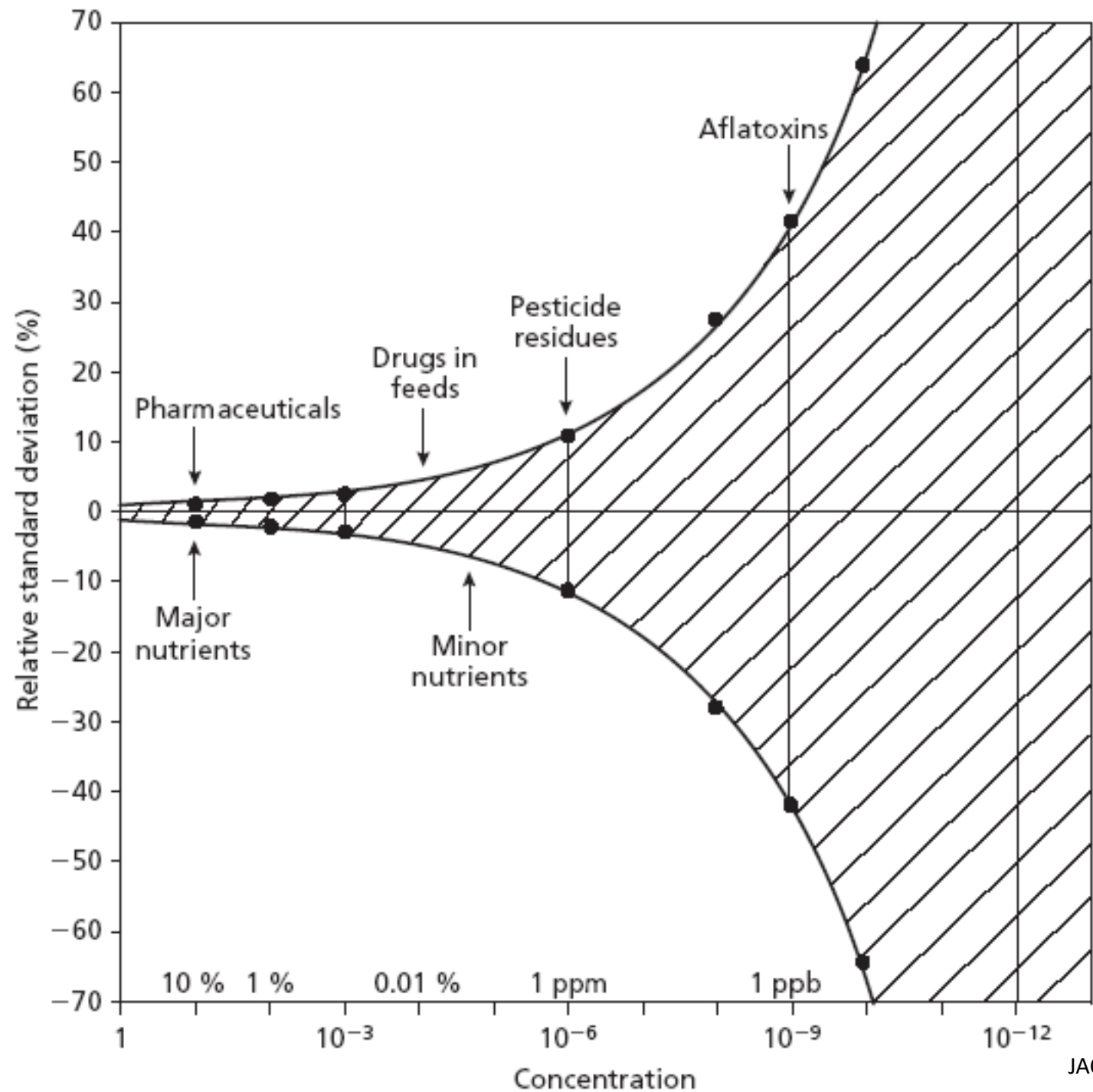
# EU Rapid Alert System for Food and Feed 1/1/20 to date

- 473 total alerts for herbs and spices
- 50 for mycotoxins (11%)
- 34 for aflatoxin
- 15 for ochratoxin
- commodities: pepper, chili, nutmeg, ginger, paprika
- India, Indonesia, Morocco, Pakistan, Peru
  
- USA and Canada



## From CODEX 15<sup>th</sup> session May 2022

- EU regulation for aflatoxin in spices 10 ppb, for ochratoxin A 10 or 20 ppb, depending on product.
- *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).





## Mycotoxin Sampling Tool (Version 1.1)

Instructions

Edit Plans

Chart Results

Table Results

Plan Summary

Export To Excel

### Instructions

Designing effective sampling plans for mycotoxin detection in food commodities is a complex task.

This **Mycotoxin Sampling Tool** provides support in analysing performance of sampling plans, and determining the most appropriate plan to meet user's defined objectives:

- The user can evaluate the effect of varying sampling plan design parameters, such as sample size, on the performance of the sampling plan.
- Using the performance information, the user can determine the most appropriate mycotoxin sampling plan to minimize risk of misclassifying lots considering available resources.

The [USER GUIDE](#) provides step by step guidance on how to use the **Mycotoxin Sampling Tool** in 26 mycotoxin-commodity combinations.

Additional references on related topics can be found on the web at <http://www.bae.ncsu.edu/usda/www/whitaker1.htm>.

## Edit Plans

Select a Mycotoxin and Commodity: OTA, Ginger, Powdered in Capsules

### Common Parameters

#### Allowable Range

#### Value

#### Kernel Count Per g:

This parameter is not applicable to ginger because the product is in a powder form.

N/A

N/A

#### Regulatory Limit (ng/g):

The regulatory limit is a maximum level established by regulatory agencies, international organizations such as Codex, or industry groups. The regulatory limit defines the difference between good lots from bad lots. If exporting to several different countries, an exporter may have to design sampling plans for each importing country due to varying regulatory limits. This will require the use of different accept/reject limits as well.

(1-100)

#### Analytical Variance Type:

Research studies have shown that among lab analytical variability is larger than within lab analytical variability. When sampling plans are designed for an individual company or processor where a single lab is used to analyze a commodity for a mycotoxin, the "within lab" option should be selected. When multiple labs are used to analyze a commodity for a mycotoxin in an industry wide program, the "among lab" option should be selected. The among-lab analytical variance is approximately double the with-in lab analytical variance. Select the Analytical Variance Type to apply using the dropdown list.

## Plan Specific Parameters

Allowable  
Range

Plan 1 ✕

Add a Plan

### Laboratory Sample Size - ns (g):

The laboratory sample is defined as the smallest size sample in g taken from the lot that is ground in a mill for sample preparation. If the user wishes to evaluate the effects of more than one sample size, the user can click on the box "Add a Plan". Up to 10 different laboratory sample sizes can be entered into the various sample size boxes. The **Mycotoxin Sampling Tool** converts the sample size in g to number of particles by multiplying the sample size in g times the kernel count per g.

(0.005-100)

5

### Number Laboratory Samples - scnt (#):

The number of laboratory samples is an important design element when evaluating the performance of attribute-type sampling plans. With an attribute type sampling plan, all sample test results have to test less than the accept/reject limit to accept the lot. There is no averaging of sample test results.

(1-300)

1

### Test Portion - nss (g):

This parameter is not applicable since the mycotoxin is extracted from the entire laboratory sample without a sample preparation step. There is no further comminution of sample.

N/A

N/A

### Number of aliquots - na:

An aliquot is defined as a specific volume taken from the solvent/test portion blend specified by the analytical method used to quantify the mycotoxin concentration in the test portion. If more than one aliquot is specified, the tool assumes that all aliquot measurements are averaged in the quantification process.

(1-300)

1

### Accept/Reject Limit (ng/g):

The accept/reject limit may or may not equal the regulatory limit. Often buyers of a commodity require that the seller use an accept/reject limit below a regulatory limit when testing a commodity before shipment to the buyer.

(0-500)

10

# From CODEX 15<sup>th</sup> session May 2022

|      |              |   |    |    |
|------|--------------|---|----|----|
| 20   | Mauritius    | All foods   | 10 |    |
| 21   | Norway       | Spices  | 10 |    |
| 22   | Pakistan     | Chilli  | 30 |    |
| 23   | Salvador     | All foods   | 20 |    |
| 24   | Singapore    | All foods except food for infants or young children | 5  |    |
| 25   | South Africa | All food stuffs                                     | 10 |    |
| 26   | Sri Lanka    | All foods   | 30 |    |
| 27   | Switzerland  | Spices excluding Nutmeg                             | 10 | 20 |
|      |              | Nutmeg  | 20 |    |
| 28   | Thailand     | All foods   | 20 |    |
| 29   | Turkey       | Spices  | 10 |    |
| → 30 | USA          | All food except milk                                | 20 |    |
| 31   | Uruguay      | All foods and spices                                | 20 |    |
| 32   | Vietnam      | All foods   | 10 |    |

“Aflatoxins (i.e., aflatoxin B1, B2, G1 and G2) are toxic by-products of mold growth on certain agricultural commodities. Since their discovery in the early 1960’s, aflatoxins have been shown to be carcinogenic to laboratory test animals. In 1969, FDA set an action level for aflatoxins at 20 parts per billion (ppb) for all foods, including animal food, based on FDA’s analytical capability and the agency’s aim of limiting aflatoxin exposure to the lowest possible level.”

**AFLATOXIN**

| <b>Commodity</b>  | <b>Action Level (ppb)</b> | <b>Reference</b> |
|---|---------------------------|------------------|
| Animal Feeds  |                           |                  |
| Corn and peanut products intended for finishing (i.e., feedlot) beef cattle   | 300                       | CPG 683.100      |
| Cottonseed meal intended for beef, cattle, swine, or poultry (regardless of age or breeding status)   | 300                       | CPG 683.100      |
| Corn and peanut products intended for finishing swine of 100 pounds or greater  | 200                       | CPG 683.100      |
| Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry   | 100                       | CPG 683.100      |
| Corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals   | 20                        | CPG 683.100      |
| Corn, peanut products, cottonseed meal, and other animal feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known | 20                        | CPG 683.100      |
| Brazil nuts   | 20                        | CPG 570.200      |
| Foods   | 20                        | CPG 555.400      |
| Milk  | 0.5 (aflatoxin M1)        | CPG 527.400      |
| Peanuts and Peanut products   | 20                        | CPG 570.375      |
| Pistachio nuts  | 20                        | CPG 570.500      |

<https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-action-levels-poisonous-or-deleterious-substances-human-food-and-animal-feed#afla>

# From CODEX 15<sup>th</sup> session May 2022

|      |              |   |    |    |
|------|--------------|---|----|----|
| 20   | Mauritius    | All foods   | 10 |    |
| 21   | Norway       | Spices  | 10 |    |
| 22   | Pakistan     | Chilli  | 30 |    |
| 23   | Salvador     | All foods   | 20 |    |
| 24   | Singapore    | All foods except food for infants or young children | 5  |    |
| 25   | South Africa | All food stuffs                                     | 10 |    |
| 26   | Sri Lanka    | All foods   | 30 |    |
| 27   | Switzerland  | Spices excluding Nutmeg                             | 10 | 20 |
|      |              | Nutmeg  | 20 |    |
| 28   | Thailand     | All foods   | 20 |    |
| 29   | Turkey       | Spices  | 10 |    |
| → 30 | USA          | All food except milk                                | 20 |    |
| 31   | Uruguay      | All foods and spices                                | 20 |    |
| 32   | Vietnam      | All foods   | 10 |    |

mold growth on materials is “damage”

Jell-O Aw 0.98



Jam Aw 0.75



Water activity or  $a_w$  is the partial vapor pressure of water in a substance divided by the partial vapor pressure of pure water.

# CODEX ALIMENTARIUS

INTERNATIONAL FOOD STANDARDS



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the United Nations



World Health  
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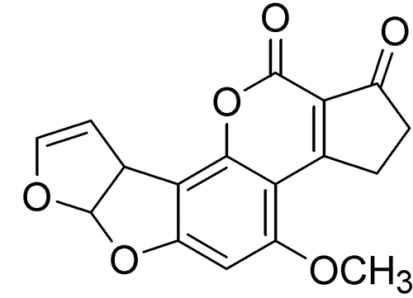
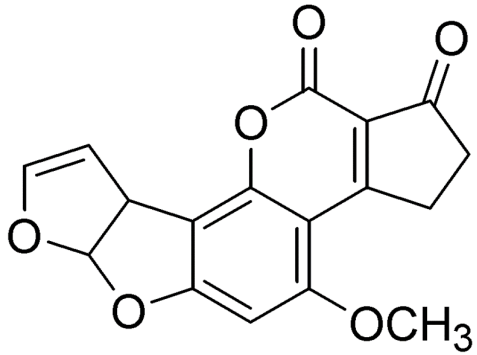
E-mail: [codex@fao.org](mailto:codex@fao.org) - [www.codexalimentarius.org](http://www.codexalimentarius.org)

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## CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF MYCOTOXINS IN SPICES

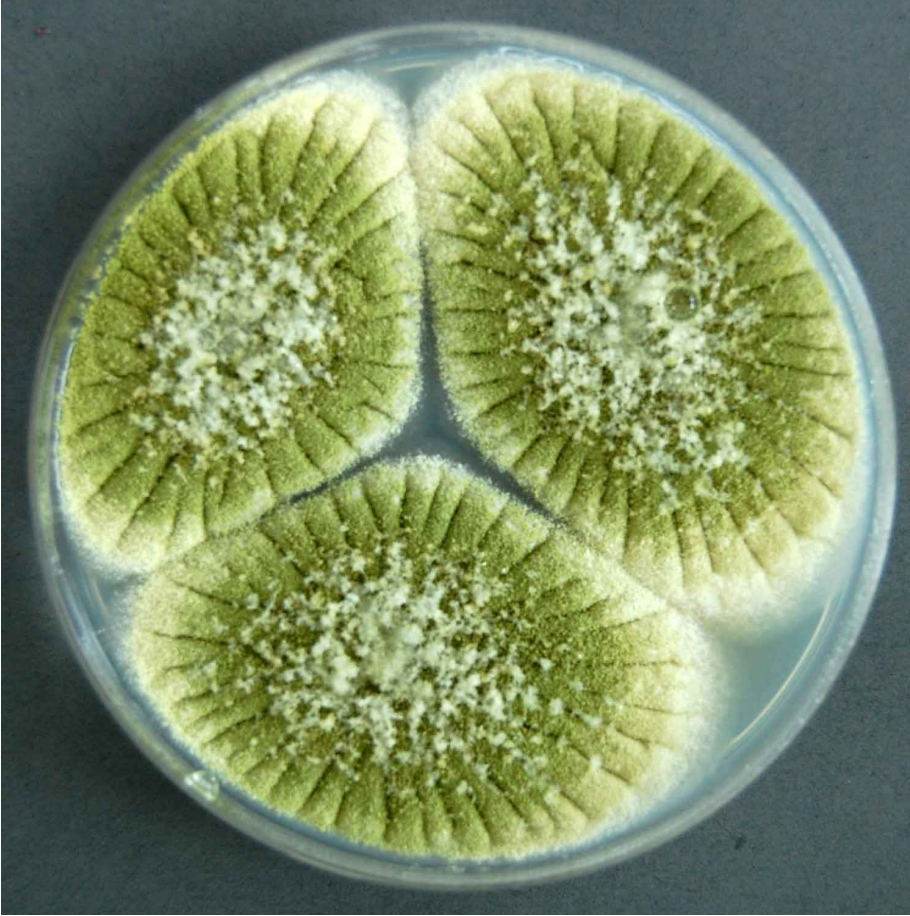
Therefore the most important point is to control the period of time in which the spices remain in the drying yard, in the range of water activity where aflatoxin and OTA-producing fungi can grow ( $a_w$  0.8–0.95). Five days or less in the drying yard is enough and effective to prevent aflatoxin and OTA accumulation. In general, a maximum  $a_w$  of 0.65 is sufficient for protecting spices from damage by fungi.

| Microfungi/Spice | <i>Aspergillus</i> spp.   |                |    | <i>Penicillium</i> spp. |      |    | <i>Fusarium</i> spp. |      |   |
|------------------|---------------------------|----------------|----|-------------------------|------|----|----------------------|------|---|
|                  | Positive <sup>a</sup> (%) | n <sup>b</sup> |    | Positive (%)            | n    |    | Positive (%)         | n    |   |
| Anise            | ●                         | 100            | 3  | ●                       | 100  | 2  | ●                    | 50.0 | 2 |
| Basil            | ●                         | 100            | 1  | ●                       | 100  | 1  | ×                    | 0.0  | 1 |
| Bay leaf         | ●                         | 100            | 2  | ●                       | 100  | 2  | ●                    | 50.0 | 2 |
| Caraway          | ●                         | 100            | 6  | ●                       | 80.0 | 5  | ○                    | 20.0 | 5 |
| Cardamom         | ●                         | 83.3           | 6  | ●                       | 50.0 | 6  | ●                    | 33.3 | 6 |
| Chili            | ●                         | 100            | 15 | ●                       | 66.7 | 9  | ●                    | 100  | 6 |
| Cinnamon         | ●                         | 50.0           | 8  | ●                       | 50.0 | 6  | ×                    | 0.0  | 5 |
| Cloves           | ●                         | 37.5           | 8  | ○                       | 14.3 | 7  | ×                    | 0.0  | 4 |
| Coriander        | ●                         | 100            | 6  | ●                       | 60.0 | 5  | ×                    | 0.0  | 5 |
| Cumin            | ●                         | 80.0           | 5  | ●                       | 75.0 | 4  | ●                    | 50.0 | 4 |
| Cumin, black     | ●                         | 100            | 2  | ●                       | 50.0 | 2  | ●                    | 50.0 | 2 |
| Curry            | ●                         | 75.0           | 4  | ×                       | 0.0  | 4  | ×                    | 0.0  | 2 |
| Fennel           | ●                         | 100            | 8  | ●                       | 50.0 | 6  | ●                    | 60.0 | 5 |
| Fenugreek        | ●                         | 100            | 3  | ●                       | 66.7 | 3  | ●                    | 33.3 | 3 |
| Garlic           | ●                         | 100            | 3  | ×                       | 0.0  | 3  | ×                    | 0.0  | 1 |
| Ginger           | ●                         | 100            | 7  | ●                       | 33.3 | 6  | ●                    | 50.0 | 4 |
| Licorice         | ●                         | 100            | 1  | ●                       | 100  | 1  | ×                    | 0.0  | 1 |
| Mace             | ●                         | 100            | 1  | ●                       | 100  | 1  | ●                    | 100  | 1 |
| Marjoram         | ●                         | 100            | 1  | ●                       | 100  | 1  | ×                    | 0.0  | 1 |
| Mint             | ●                         | 100            | 1  | ●                       | 100  | 1  | ×                    | 0.0  | 1 |
| Mustard          | ●                         | 66.7           | 3  | ●                       | 66.7 | 3  | ×                    | 0.0  | 3 |
| Nutmeg           | ●                         | 90.0           | 10 | ●                       | 60.0 | 10 | ×                    | 0.0  | 4 |
| Oregano          | ●                         | 100            | 2  | ●                       | 100  | 1  | -                    | -    | 0 |
| Paprika          | ●                         | 100            | 2  | ●                       | 100  | 1  | ●                    | 100  | 1 |
| Parsley          | ●                         | 100            | 1  | ●                       | 100  | 1  | ×                    | 0.0  | 1 |
| Pepper, black    | ●                         | 91.7           | 12 | ●                       | 75.0 | 8  | ●                    | 33.3 | 6 |
| Pepper, white    | ●                         | 100            | 6  | ●                       | 50.0 | 4  | ×                    | 0.0  | 2 |
| Rosemary         | ●                         | 100            | 3  | ●                       | 50.0 | 2  | ×                    | 0.0  | 1 |
| Saffron          | ●                         | 66.7           | 3  | ●                       | 50.0 | 2  | ×                    | 0.0  | 2 |
| Star anise       | ×                         | 0.0            | 1  | ×                       | 0.0  | 1  | ×                    | 0.0  | 1 |
| Sumac            | ●                         | 50.0           | 2  | ×                       | 0.0  | 2  | ●                    | 50.0 | 2 |
| Thyme            | ●                         | 100            | 3  | ●                       | 33.3 | 3  | ●                    | 100  | 1 |
| Turmeric         | ●                         | 100            | 5  | ●                       | 80.0 | 5  | ●                    | 60.0 | 5 |



| Spice         | <i>A. flavus</i>          |                | <i>A. parasiticus</i> |   | <i>A. niger</i> |    | <i>A. versicolor</i> |      | <i>A. ochraceus</i> |      |   |
|---------------|---------------------------|----------------|-----------------------|---|-----------------|----|----------------------|------|---------------------|------|---|
|               | Positive <sup>a</sup> (%) | n <sup>b</sup> | Positive (%)          | n | Positive (%)    | n  | Positive (%)         | n    | Positive (%)        | n    |   |
| Chili         | 90                        | 10             | 55.6                  | 9 | 90.9            | 11 |                      | 75   | 4                   | 40   | 5 |
| Cinnamon      | 33.3                      | 6              | 25                    | 4 | 66.7            | 6  |                      | 0    | 2                   | 66.7 | 3 |
| Cloves        | 37.5                      | 8              | 33.3                  | 6 | 25              | 8  |                      | 0    | 3                   | 0    | 3 |
| Ginger        | 75                        | 4              | 60                    | 5 | 60              | 5  |                      | 33.3 | 3                   | 50   | 2 |
| Nutmeg        | 66.7                      | 9              | 75                    | 4 | 55.6            | 9  |                      | 50   | 4                   | 66.7 | 3 |
| Pepper, black | 88.9                      | 9              | 75                    | 8 | 88.9            | 9  |                      | 100  | 4                   | 100  | 5 |
| Pepper, white | 80                        | 5              | 75                    | 4 | 100             | 4  |                      | 100  | 1                   | 0    | 2 |

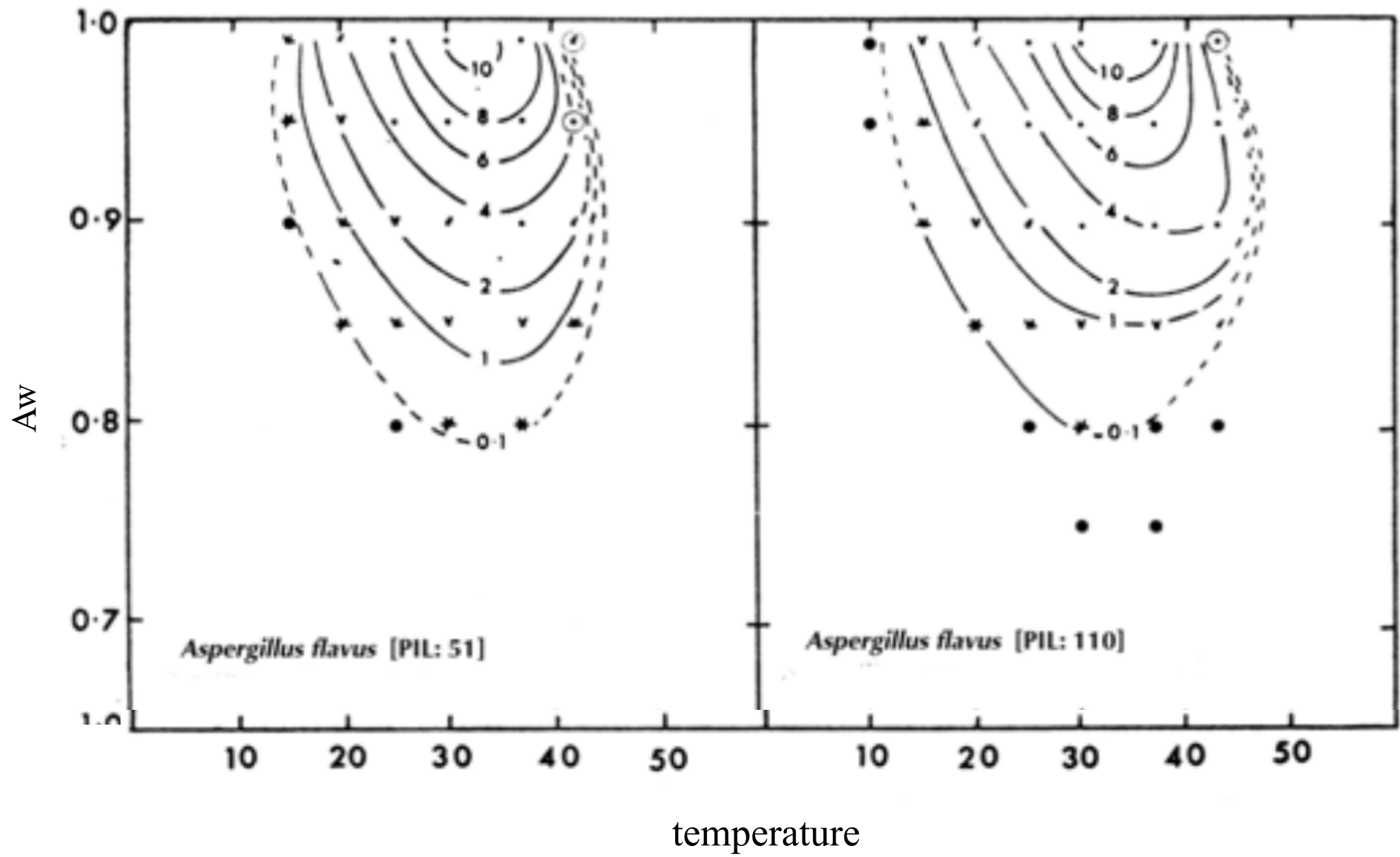
Adapted from Toxins 2020, 12, 789;

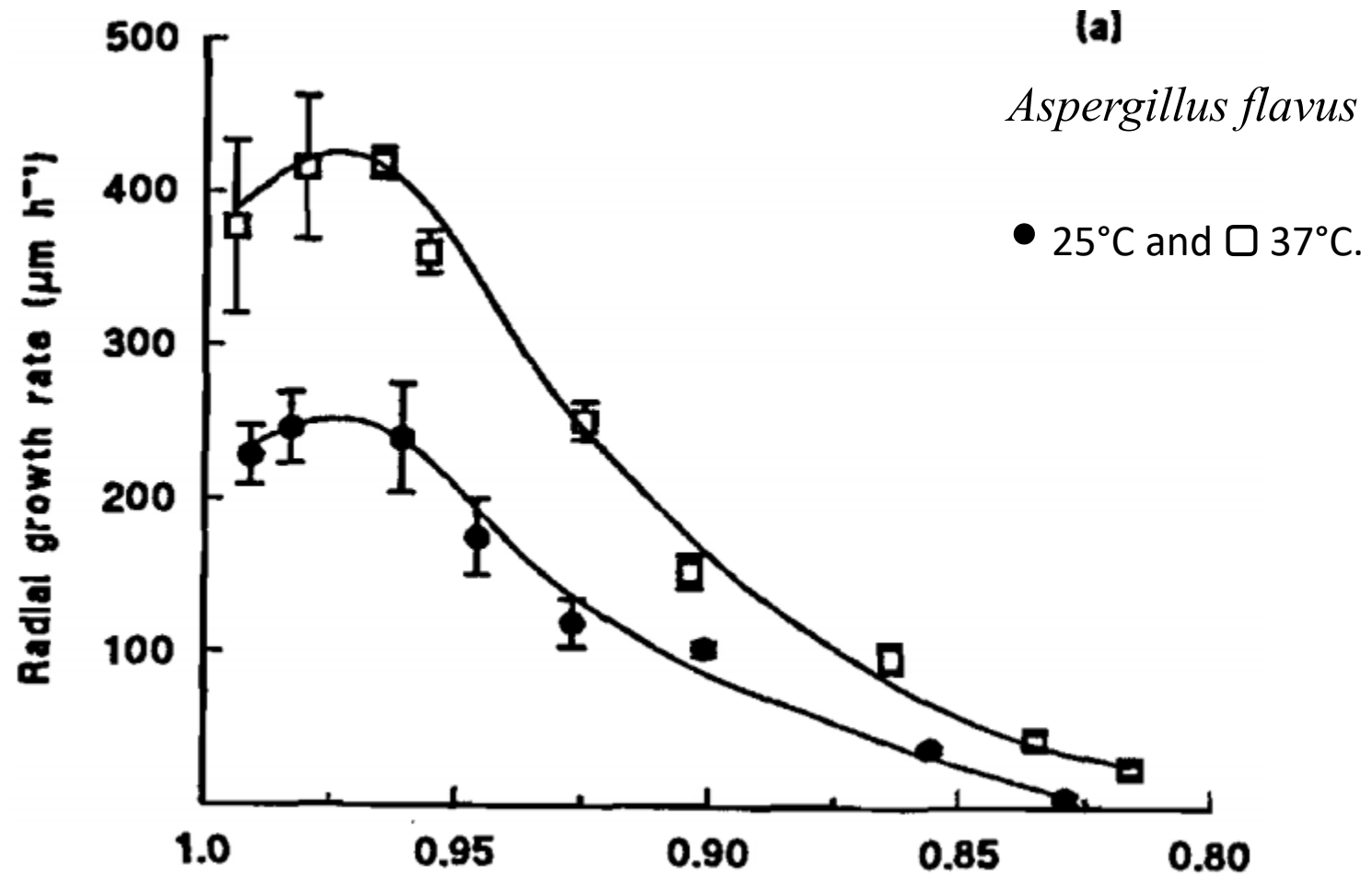


*Aspergillus flavus*

*A. parasiticus* on peanuts

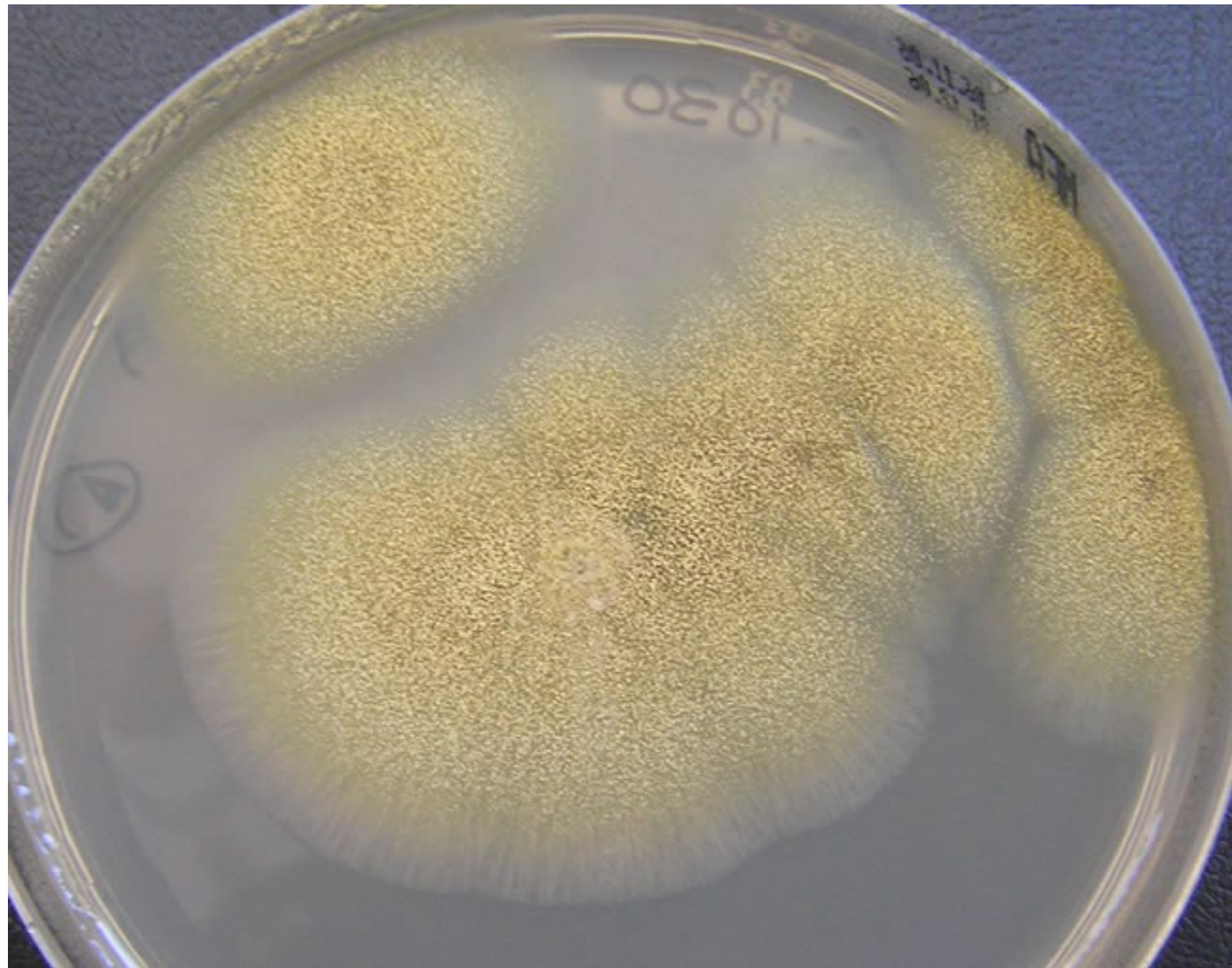
plus 11 other species





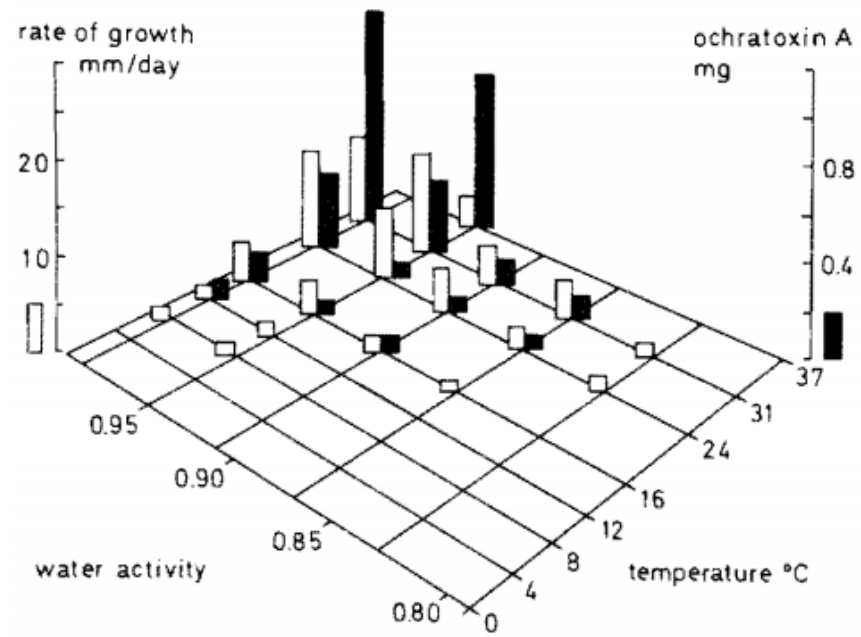
# Three complicating factors

- The quality of nutrients available in the substrate affects the ability of fungi to grow at particular  $a_w$  values. When the substrate composition includes water-extractable compounds containing nitrogen and readily metabolizable carbon, the lower limit  $a_w$  goes down.
- When fungi grow on a substrate, they excrete hygroscopic materials that change the water relations of the colonized material from the original state. After colonization becomes extensive, the fungal colony becomes as much of a determinant of the relationship between ambient moisture, absorption, and desorption as the material itself. Fungal growth also changes the nutrient status of the colonized material.
- Time of wetting can change the distribution of fungi. Some fungi are more tolerant to brief dry periods than others.



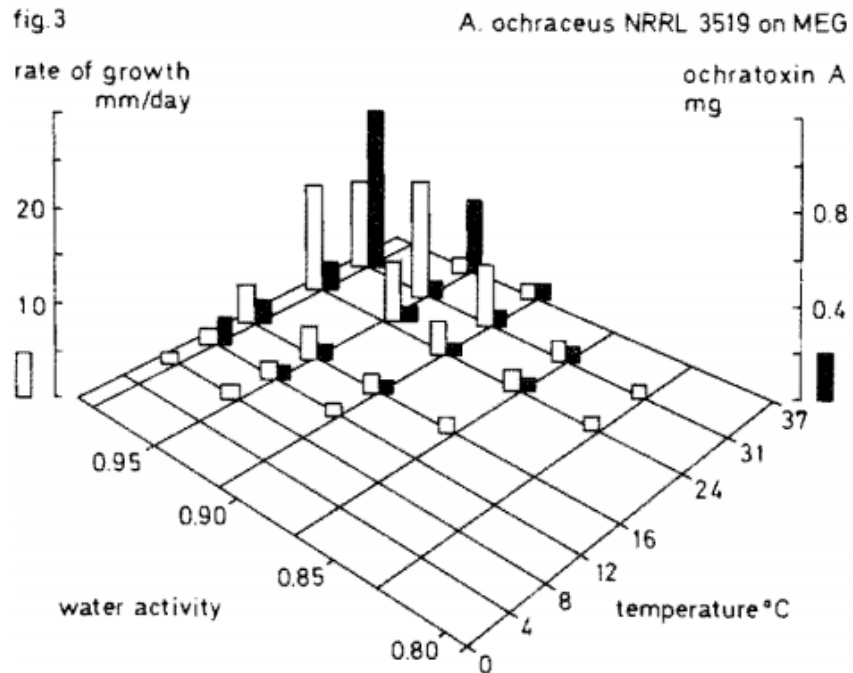
*Aspergillus ochraceus*

fig. 2



malt extract sucrose agar

fig. 3



malt extract glycerol agar

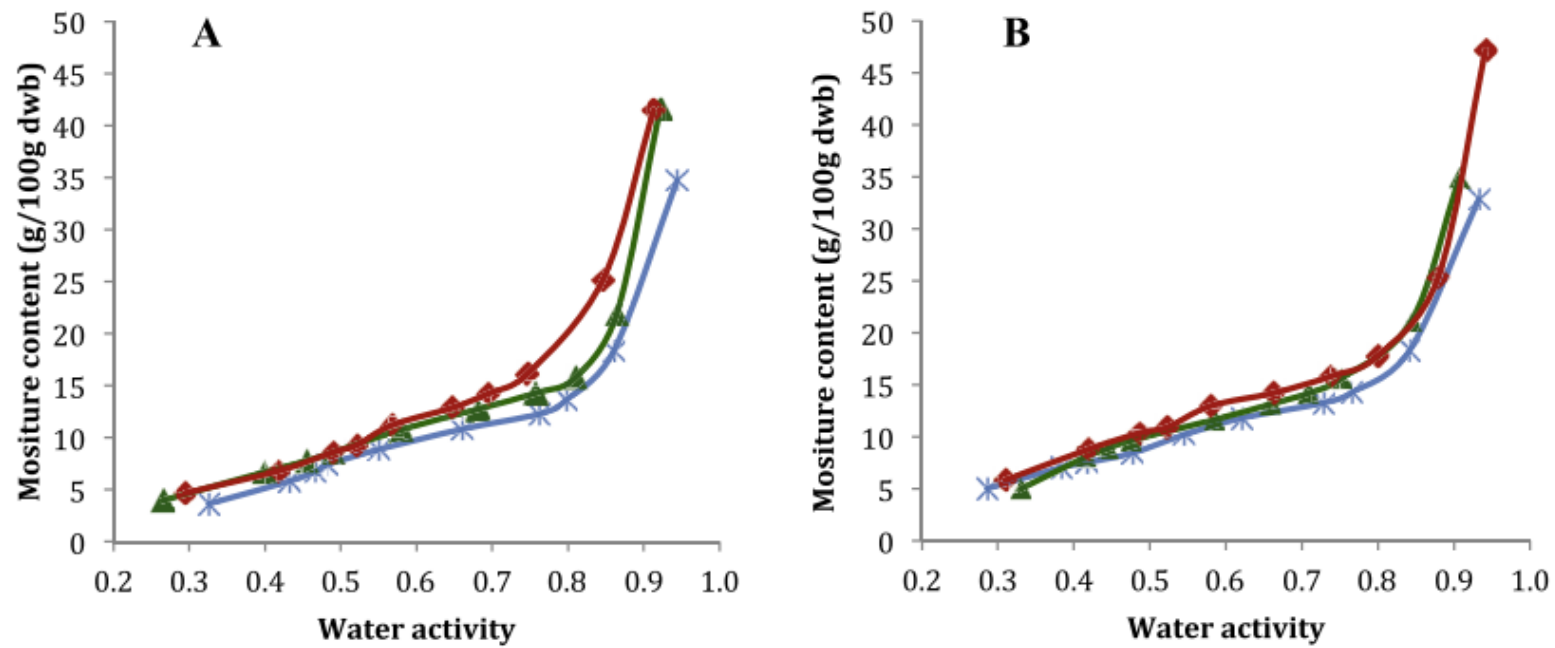


## CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF MYCOTOXINS IN SPICES

The drying process can be divided into three stages. In each stage, aflatoxin and OTA producing fungi will have varying opportunities for growth:

- ?
- (a) At the first stage, there is a slight decrease in moisture content. The high moisture content ( $a_w > 0.95$ ) provides unsuitable conditions for aflatoxin and OTA producing fungi to grow. However, other microorganisms, such as other hydrophilic fungi (yeasts and moulds) and bacteria, may spoil the product if it is kept too long at  $a_w > 0.95$  after harvest.
- (b) The second stage is the one of maximum loss in moisture content. During this stage ( $a_w$  lower than 0.95 but higher than 0.80), there are favourable conditions for aflatoxin and OTA producing fungi to grow and therefore it is necessary to implement precautionary measures as recommended in paragraphs 28 to 33.
- (c) The third stage which starts at  $a_w 0.80$ , is much drier compared to the previous two stages. There is a slower slight decrease in the remaining moisture content. Conditions at this stage do not favour the growth of aflatoxin and OTA producing fungi.

Therefore the most important point is to control the period of time in which the spices remain in the drying yard, in the range of water activity where aflatoxin and OTA-producing fungi can grow ( $a_w 0.8-0.95$ ). Five days or less in the drying yard is enough and effective to prevent aflatoxin and OTA accumulation. In general, a maximum  $a_w$  of 0.65 is sufficient for protecting spices from damage by fungi.



**Fig. 1.** Adsorption (A) and desorption (B) isotherms of whole black peppercorns at temperatures,  $\blacklozenge$  22 °C,  $\blacktriangle$  30 °C and  $\ast$  37 °C.

Aw is the biologically available water

chemically bound

free water

accuracy really matters

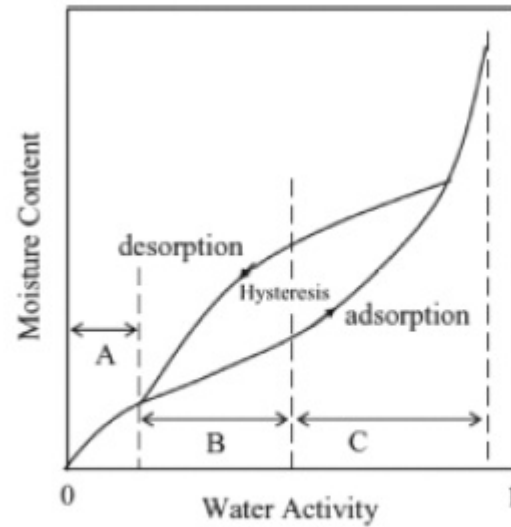
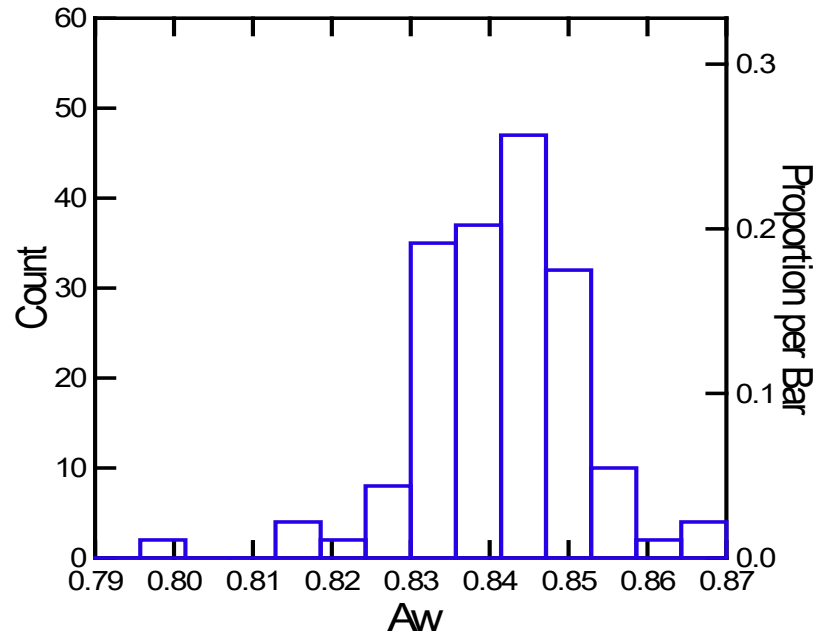
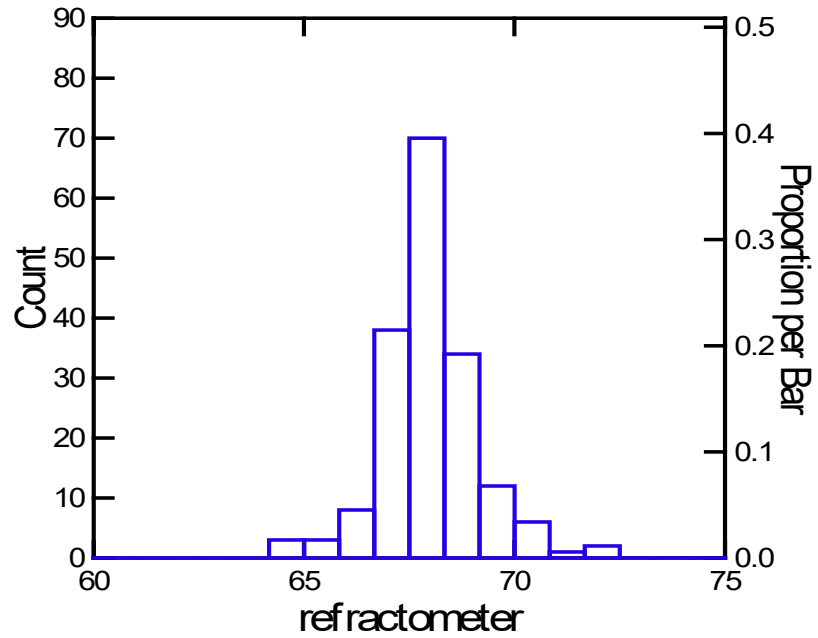
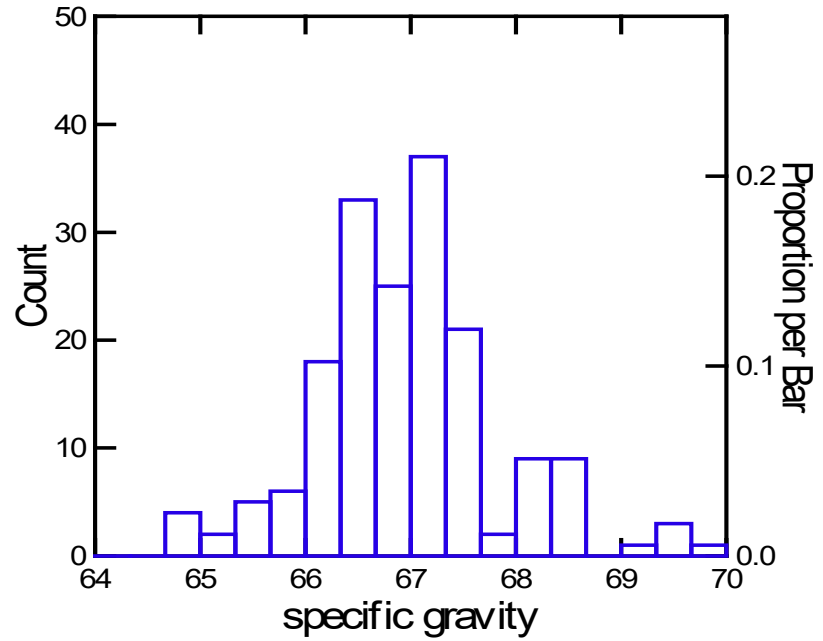
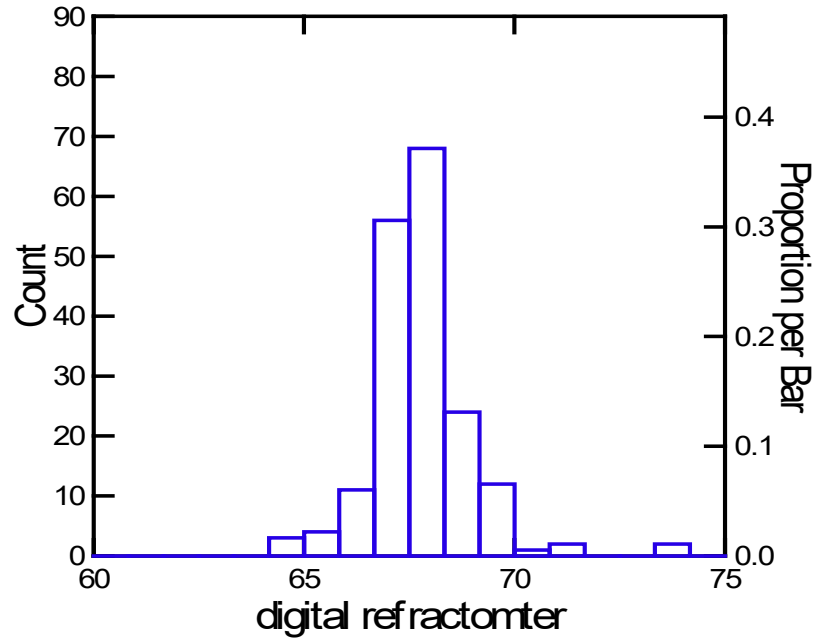


Figure 1. Sorption isotherm for a typical food product, showing the hysteresis.





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## CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF MYCOTOXINS IN SPICES

Therefore the most important point is to control the period of time in which the spices remain in the drying yard, in the range of water activity where aflatoxin and OTA-producing fungi can grow ( $a_w$  0.8–0.95). Five days or less in the drying yard is enough and effective to prevent aflatoxin and OTA accumulation. In general, a maximum  $a_w$  of 0.65 is sufficient for protecting spices from damage by fungi.



Roles of company directors and the implications for governing for the emerging impacts of climate risks in the fresh food sector: A review<sup>\*</sup>

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“A review of the impact of climate change on chemical exposures affecting human health identified 8 contaminants as high risk of getting worse due to climate change, one of which was mycotoxins (Balbus et al. 2013). Guerin (2022) evaluated the risks and responsibilities of Directors of companies that supply food in relation to climate change. One of the risks flagged was mycotoxins. Key responsibilities included (1) encouraging collaboration across supply chains, (2) understand the effectiveness of existing controls and if necessary, seek external advice, and (3) invest in new technology to better manage the risk of climate change for the company and consumers.”