LGC AXIO PROFICIENCY TESTING

Unmasking Mycotoxins in Spices

AXIO investigates chemical and microbial hazards in spices. Including; occurrence, risk, testing and lessons from PT.

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ISO/IEC 17043

Introduction

Spice products face varying potential concerns, mainly relating to microbial and chemical contaminants. Microbial contaminants of note include pathogens, hygiene indicators and spoilage organisms, whilst chemical contaminants of concern include heavy metals, dioxins, pesticide residues, and mycotoxins. There are also other considerations that require stringent monitoring, including, but not limited to, authenticity, adulteration, and addition of unauthorised colourings. The wide scope of risks to consumer safety and brand integrity highlights the importance of rigorous and thorough analytical testing of spices.

This paper will focus on mycotoxins. Although mycotoxins are considered a chemical contaminant, they are produced by the growth of mould. Mycotoxins are persistent as even if the mould is destroyed during the processing of spices, the mycotoxin may remain..

Drawing upon over 40 years of proficiency testing experience, we will review the challenges to analytical laboratories testing responsible for mycotoxin analysis in spices, as well as a review of testing methods in spices. Finally, we will review our own laboratory data from our Quality in Food Chemistry Scheme (QFCS) to review best practices in mycotoxin analysis.

Defining a spice

A spice is a seed, fruit, root, bark, or other plant substance primarily used for flavouring or colouring food. Additionally, the physical and chemical properties of spices means that they have a diverse range of uses outside of food, including medicine, religious rituals, cosmetics, and perfume production. There are roughly 40 different spice (and herb) plants of global importance, and many more with local relevance [1]. Examples of spices include garlic, ginger, cinnamon, pepper, turmeric, cardamon, cumin, etc. Spices may be prepared and available in several forms: fresh, whole dried, or pre-ground dried, depending on the nature of the material and its typical uses.

An overview of Chemical hazards found in spices

Chemical hazards commonly found in spices include heavy metals, mycotoxins, PAHs, illegal dyes, or pesticide residues.

A hazard in this case, is a chemical compound or a group of compounds that have the potential to cause harm.

A risk is the likelihood of harm taking place therefore the presence of a hazard does not necessarily mean that there is a risk associated with the consumption of these food products.

Foods like spices are usually consumed in small quantities, hence they represent a small percentage of total diet and minor addition to the total daily dose of a particular contaminant. This is of course, subject to various cooking/consumption habits in different cuisines around the world. Appropriate risk assessments must be conducted to ensure that the risk is properly calculated.

Contaminants can be defined as substances (hazards) that have not been intentionally added into food. The way a product is processed or produced can lead to the formation of chemicals entering the food during manufacturing, handling, storage, processing, or distribution. Some contaminants may also enter the food chain from the environment, for example through contaminated water or mineral rocks (e.g. heavy metals). The presence of such substances in food must be monitored to ensure the safety and quality of the food we consume.

On the following page we give an overview of chemical contaminants frequently found in spices...

An overview of Chemical hazards found in spices (continued)

Lead contamination

There are increasing concerns over lead (Pb) toxicity, especially in children, as a direct result from spice consumption. Pb can contaminate spice products at multiple points throughout the farm to fork process. The US Environmental Protection Agency recently stated that that the consumption of spices in children is generally low in the US (0.09g/day for cumin). However, a recent survey in North Carolina which investigates lead exposure in children with elevated blood lead levels concluded that the consumption patterns differ depending on the cultural background of their parents. For example, it was estimated that children whose parents emigrated from Southeast Asia may consume up to 1.22 ± 1.14 g per portion of cumin daily. [2] The survey also reports that some spices like turmeric, may contain up to 740mg/kg of Pb. [2]

Pesticide residues

Pesticide residue are a cause for concern in all spices. In paprika for example, the European Food Safety Authority (EFSA) pesticide report, 2020, stated that 23% of paprika powder samples tested had a high Maximum Residue Limit (MRL) exceedance rate, and a further 0.3% of paprika samples had the highest frequency of multiple MRLs. Meanwhile in Stuttgart, Germany, the CVUA* analysed 20 samples of paprika powder for the presence of pesticide residues. Nineteen (95%) samples were found to contain up to 40 pesticides in concentrations exceeding the legally established MRL. [3]

***CVUA is the European Union Reference Laboratory-EURL- for "Residues of pesticides food of animal origin and commodities with high fat content"**

Adulteration and fraud

Increasing global demand and the complexity of the global food supply chain, offers greater opportunities for criminals to commit fraud with relatively low probability of being caught. Herbs and spices are included in the list of the most commonly targeted products.

During an EU coordinated control plan (2021) for the adulteration of fraud in herbs and spices, 21 EU member states, plus Norway and Switzerland, submitted 1900 samples for analysis. The analysis concluded that 17% of pepper samples, 14% of cumin, 11% of curcuma, and 11% of saffron were considered suspicious for adulteration. The overall rate of suspicious samples was 17%, which translates to 323 of 1885 analysed samples. [4]

Polycyclic Aromatic Hydrocarbons

Polycyclic Aromatic Hydrocarbons (PAHs) are a complex class of toxic organic pollutants, some of them like Benzo[a]pyrene are classified as a human carcinogen by the International Agency for Research on Cancer. A 2015 survey conducted by FERA** for the Food Standards Agency in the UK, reported that the concentrations of the 4 European Union (EU) marker PAHs (benz[a] anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene) in herbs and spices ranged from 0.44 µg/kg in ground coriander, to 74.21 µg/kg in whole cloves, with a mean of 17.76 µg/kg. [5]

****FERA is the UK National Reference Laboratory for PAHs in food**

Illegal dyes

Synthetic dyes have been widely used in the spice industry as colouring agents. The use of many of these compounds is now forbidden in food products as they have the potential to cause adverse effects to human health. Some compounds include Sudan dyes, tartrazine, azorubin, bixin, and many more. The presence of non-authorised dyes was included as part of the EU coordinated control plan (2021) mentioned above. The overall spices adulterated by dyes was 2% of samples, which translates to 25 out of 1340 samples tested. Saffron had the highest percentage of samples (8.5%) containing non-authorised dyes, which was 12 out of 141 samples tested.

A focus on Microbiology

As spices are produced from plants, they are subject to several processes between field to fork and if not handled correctly, microbiological contamination could occur at any stage. Microbiological contamination may be introduced during cultivation, harvesting and/or processing and can come from several possible sources, including soil, air, water, insects, other animals, fertilisers, or human handlers.

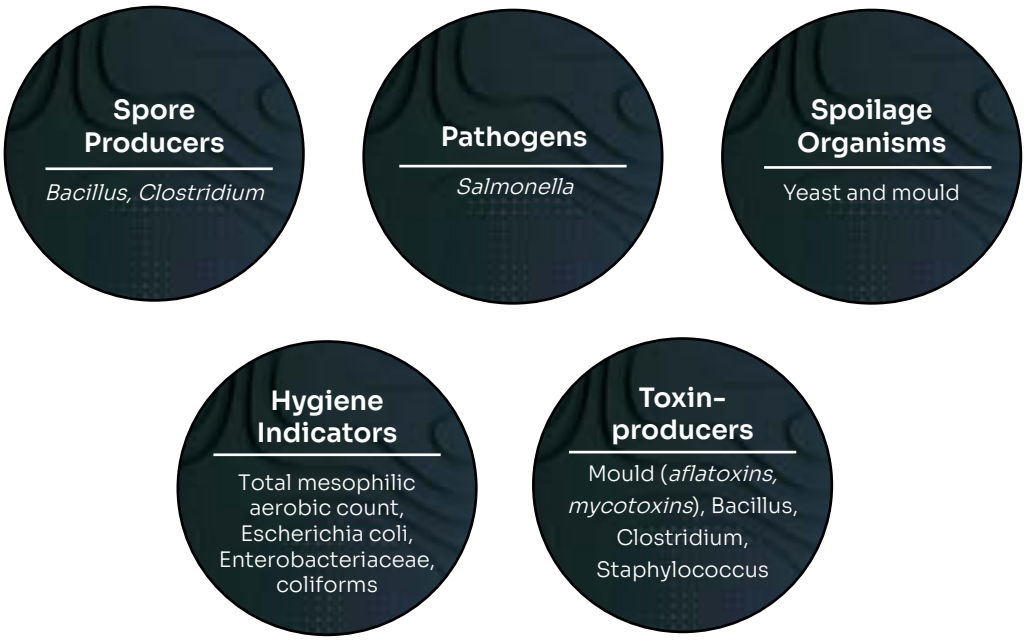
Spices themselves do not provide an ideal environment for growth of microorganisms, due to their low water content and high osmotic pressure. Some spices are also known to have anti-bacterial properties, [6] such as garlic and ginger, and there are examples of these being used in other foods, particularly meats, in order to help preserve them, such as garlic being added to chorizo sausages. The use of garlic as an antimicrobial agent can be traced as far back as 2000BC where it has been recorded as being used in traditional Chinese medicine.

Despite the adverse conditions for growth, microorganisms are well known for their adaptability, and there are many examples where they have been found to thrive very well in spices in relatively high numbers for long periods of time. Due to the need to maintain the aroma and flavour, spices cannot be preserved by traditional methods such as pasteurisation or use of preservatives, which means that if undesirable microorganisms are introduced at any stage, it can be difficult to remove them.

Microorganisms in spices

Spices can become contaminated with a variety of microorganisms, but the most common are organisms that can survive in an environment with low water levels, such as moulds and spore-forming microorganisms such as Bacillus and Clostridium species.

Microorganisms of interest in spices are;



A number of studies have shown that commercially available spices can contain a range of microorganisms at relatively high levels. For example, a study to perform microbial profiling of spices in Ghana found almost all samples tested contained coliforms. Studies, including [7], carried out worldwide, have found spice samples contaminated with all of the microorganisms listed above.

Implications

To preserve the aroma and taste, spices are often added to foods at the last-minute during cooking and may also be added to ready-to-eat foods with no further processing. The cooking process cannot therefore be relied upon to destroy any microorganisms that may be present.

Although usually used sparingly, even small quantities of contaminated spices do have the capacity to cause illness. The European Rapid Alert System for Food and Feed (RASFF) regularly reports alerts on spices. Between 2008 and 2011, 22 alerts relating to spices were reported, of which 21 were due to Salmonella species and Escherichia coli. A report [8] on the implication of spices in cases of outbreaks or disease caused by microbial contamination found that the commonest bacteria found in various food incidents were as listed below;

- *Bacillus cereus*
- *Cronobacter* species
- *Clostridium botulinum*
- *Clostridium perfringens*
- *Escherichia coli*
- *Verotoxic Escherichia coli*
- *Listeria monocytogenes*
- *Salmonella* species
- *Staphylococcus aureus*

There are a number of examples of outbreaks of foodborne illness [9] due to spices, with one of the largest occuring in 2009-10 in the USA, where 272 people across 44 states were infected with Salmonella from black and red pepper used in a ready-to-eat salami product.

Microbial criteria

Microbiological criteria can be used at all stages in the food chain, from raw materials to finished product, and define what is considered acceptable in terms of microbiological content. They are based on the absence/presence or levels of particular microorganisms, per sample based on a defined mass, volume, lot, etc. Microbial criteria may be mandatory, for example specified by governments and written into regulations, or may simply be available as guidelines or agreed on a case-by-case basis between buyers and producers.

Examples of microbiological criteria for spices are shown in the table below:

Classification of dried herbs and spices from retail premises as recommended by microbiological criteria within Recommendation 2004/24/EC and ESA

Microorganism	Microbiological quality (colony forming units (cfu)/g unless stated)		
	Satisfactory	Acceptable	Unsatisfactory
Bacillus cereus	$<10^3$	10^3 to 10^4	$> 10^4$
Clostridium perfringens	$<10^2$		$> 10^3$
Salmonella species	Not detected in 25g		Detected in 25g
Escherichia coli	$<10^1$		$> 10^2$

Microbial Testing

Several issues present a challenge in the microbiological study of dried herbs and spices. Dryness, inhibiting substances, high osmotic pressure, and other adverse conditions heavily stress the cells. Test methods therefore need to consider the likely presence of stressed cells and use appropriate techniques to encourage recovery. In most cases the same procedures as for a range of other food and dairy products can be used, however there are some specific ISO standards for preparation and testing of products likely to contain stressed cells.

ISO 6887-4 :2017 – Microbiology of the food chain — Preparation of test samples, initial suspension, and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of miscellaneous products

ISO 6887-4:2017 is applicable to a large number of products, such as acidic (low pH), hard and dry products, animal feed, margarines etc and also dehydrated, freeze-dried and other low Aw products (including those with inhibitory properties) which would apply to testing of spices.

ISO 21527-2:2008 - Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0.95. This standard applies to dry fruits, cakes, jams, dried meat, salted fish, grains, cereals and cereals products, flours, nuts, spices and condiments, etc.

Prevention of contamination

Prevention of microbial contamination in spices lies in the application of good hygiene practices during growing, harvesting and processing from farm to fork, particularly during the drying process, which is often done by laying out the spices in the sun rather than under controlled conditions. Methods for microbial control and decontamination during processing may include steam treatment, irradiation, Ref F chlorination and vacuum-packing.

In addition, the importance of correct food handling practices and usage of spices by end users cannot be overemphasised. As dried products with a relatively long shelf-life, spices can often be inadvertently kept in cupboards well beyond their shelf-life. End-users should check that spices are kept in a dark, dry environment in well-sealed containers and avoid contamination from moisture, other foods or from users.

Proficiency Testing (PT) scheme for microorganisms in spices

The LGC PT schemes provide a comprehensive range of products that are often presented in a generic food material such as oatmeal, in order to represent a wide range of different foodstuffs. However, in order to provide a realistic challenge to reflect the unique properties of spices, LGC also offers a number of microbiology PT samples specifically in a spice matrix. A summary of PT results for samples and analytes in spices are given in the Table below, showing the total number of results over 5 years, the analyte under test, the assigned value (best estimate of level of organism in the sample) and standard deviation for each test, and the % of participants obtaining satisfactory results;

Analyte	Number of results	Average Assigned Value (log10)	Standard Deviation of Results (log10)	% Satisfactory results
Total aerobic mesophilic count	308	4.79	0.26	94.4
Coliforms	310	4.06	0.33	92.9
Enterobacteriaceae	241	4.08	0.28	96.6
Escherichia coli	292	4.06	0.35	94.6
Bacillus cereus	201	4.20	0.40	88.2
Coagulase positive staphylococci	318	4.00	0.35	95.6
Yeast	278	3.63	0.26	96.9
Mould	280	3.29	0.36	92.7
Yeast & Mould	163	3.82	0.28	98.3

The performance of participants testing spices as a matrix is fully comparable to that of participants carrying out PT using other matrices and provides a useful way for participants to compare their performance and methods with other laboratories testing spice products.

A focus on mycotoxins

Mycotoxins are naturally occurring, low-molecular-weight compounds, produced as secondary metabolites by more than 100 species of filamentous fungi mainly *Aspergillus, Penicillium* and *Fusarium.* The word mycotoxin is derived from the Greek words “mykes” and “toxini”, meaning fungus and toxin, respectively.

The type of microorganism which grows on a crop or product is affected by the matrix, and environmental conditions like temperature and humidity, both during cultivation, production and during storage. Temperature and humidity are important parameters for the growth of fungi, therefore, climate change may also play a role in the presence of mycotoxins in food and animal feed. [6]

Mycotoxins do not form a toxicologically and chemically homogeneous group, though rather they are grouped together only because the members can cause disease and potentially death in human beings and other vertebrates.

Mycotoxin types and prevalence in crops and spices

Aspergillus flavus and *Aspergillus parasiticus* grow in soil and decaying vegetation.

A.flavus and *A.parasiticus* mainly produce Aflatoxins as secondary metabolites. *A. flavus* produces the B-series aflatoxins, while *A. parasiticus* produce both B- and G-series. The "B" and "G" describe the colour each compound fluoresces in UV light, which is Blue and Green respectively. The subscript numbers indicate major (1) and minor (2) compounds [11].

Crops that are frequently affected by *Aspergillus* spp. include cereals like corn or rice, groundnuts and oilseeds like peanut or cotton seeds, spices like chilli peppers, black pepper, ginger, and tree nuts like pistachio nuts and almonds.

Several species of *Aspergillus* and *Penicillium,* also produce Ochratoxin A (OTA).

Fusarium fungi are also commonly found in the soil and produce a range of different toxins, including trichothecenes such as deoxynivalenol (DON), nivalenol and T-2 and HT-2 toxins, as well as zearalenone (ZON or sometimes abbreviated as ZEN) and fumonisins.

Penicillium, Aspergillus and *Byssochlamys* may also produce the mycotoxin patulin but this occurs mainly in apples or in apple juice and products that contain apples.

Aspergillus, Penicillium, and *Monascus* fungi may produce citrinin, another mycotoxin that occurs often in stored grains, herbs and spices. *Alternaria spp*. produce more than 70 secondary metabolites and some of them are classified as mycotoxins e.g., alternariol. [12]

The genus *Claviceps*, mostly *Claviceps purpurea* produce ergot alkaloids (EAs), including ergometrine, ergosine, ergocornine and ergotamine. According to EFSA, the highest levels of EAs were detected in rye and rye containing products. [13]

Regulations

The European Commission (EC) established the most thorough and comprehensive legislation for mycotoxin in food and in feed [14], but other countries like USA, Japan or Brazil have their own legislation, generally less comprehensive and less restrictive.

The International Codex Committee on Contaminants in Food (CCCF) establishes permitted maximum levels or guideline levels for contaminants and naturally occurring contaminants in food and feed. In addition, priority lists of contaminants are prepared for risk assessment by the Joint FAO/WHO Expert Committee on Food Additives (JEC-FA).

Specifically for spices, CODEX has adopted a "Code of practice for the prevention and reduction of mycotoxins in spices, CXC 78-2017 [15]. This Code should be used in conjunction with the Code of Hygienic Practice for Low Moisture Foods (CXC 75-2015) and its Annex on spice and culinary herbs, and other relevant Codex codes of practice.

CODEX has also produced standards for chilli peppers, black, white, and green pepper, cumin, dried roots, rhizomes and bulbs specifically for dehydrated ginger, and cloves but they are mostly related to the quality and essential composition of the spices concerned. [16]

The contaminants are covered under a General CODEX standard for contaminants and toxins in food and feed, CXS 193-1995 revised in 2019 [17]. There is an ongoing discussion at CODEX level to establish maximum limits in spices and it seems like the levels for discussion are around the ball-park of 20-30 µg/kg for total aflatoxins and 20 µg/kg for OTA. [18]

Some information related to mycotoxins in spices across the European Union, Japan and the United States of America are shown below.

Matrix	Mycotoxin	Regulation
Spices, including dried spices *Piper* spp. *Myristica fragrans* (nutmeg) *Zingiber officinale* (ginger) *Curcuma longa* (turmeric)	Ochratoxin A: 15 µg/kg Aflatoxin B1: 5 µg/kg Total Aflatoxins (EU & Japan-all food: 10 µg/kg Total Aflatoxins (US-all food): 20 µg/kg	EU Commission Regulation (EC) No 1881/2006 of 19 December 2006 as amended on 01/07/2022 US Federal Food, Drug and Cosmetic Act (FFDCA)
Capsicum spp. (dried fruits thereof, whole or ground, including chillies, chilli powder, cayenne and paprika)	Ochratoxin A: 20 µg/kg Aflatoxin B1: 5 µg/kg	EU Commission Regulation (EC) No 1881/2006 of 19 December 2006 as amended on 01/07/2022

Exposure to mycotoxins

Exposure to mycotoxins may occur as a results of eating contaminated foods or from animals that were fed contaminated feed. The most common mycotoxins which may pose a concern to human or animal health include aflatoxins, ochratoxin A and deoxynivalenol.

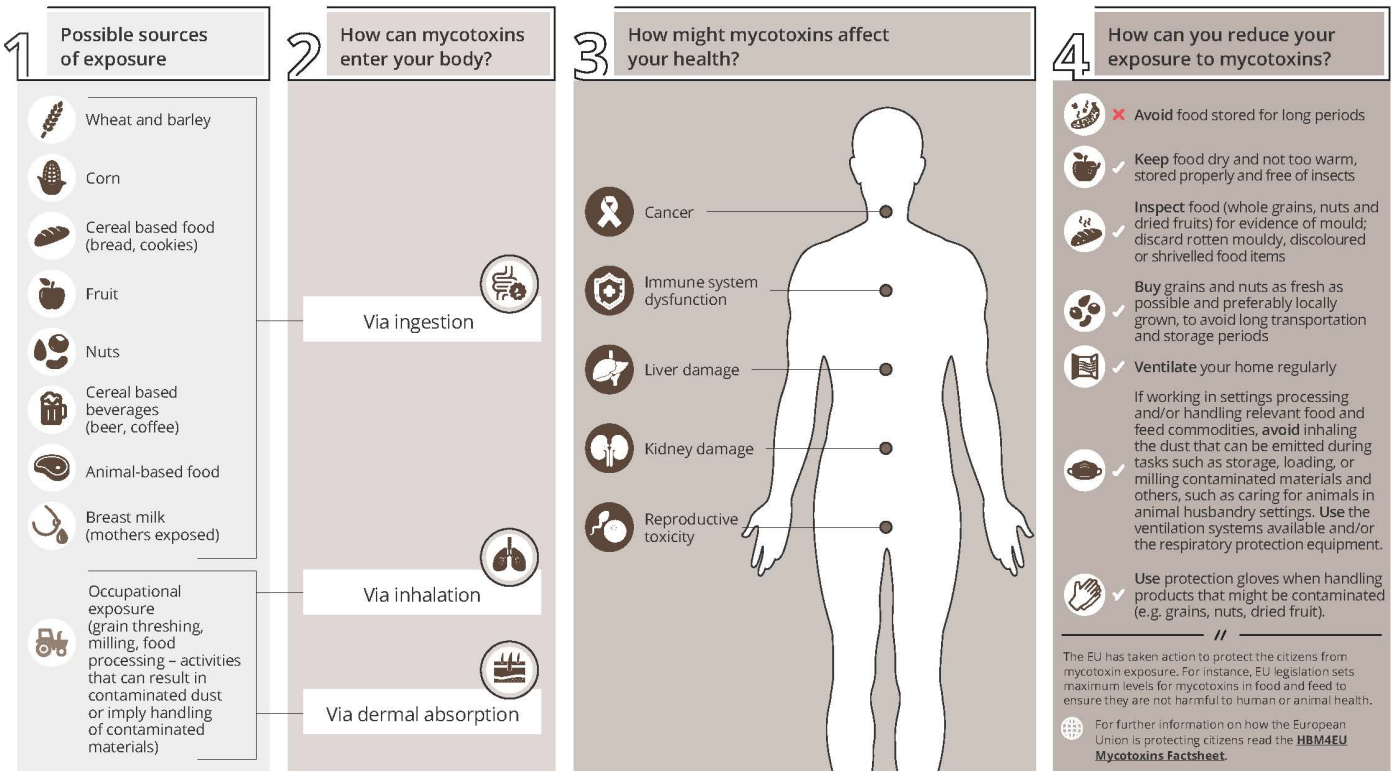
Aflatoxin B1 is identified as potent human carcinogen by the International Agency for the Research on Cancer (IARC). Other mycotoxins are suspected or known to be carcinogenic or to have other adverse health effects.

The mycotoxins infographic in figure 1, shows possible routes of exposure, the way mycotoxins enter the body, how they can affect health and ways to reduce the exposure to mycotoxins.

Non-compliance

The economic losses associated with mycotoxin contamination are difficult to assess in a consis-tent and uniform way, but they affect the whole of the supply chain.

In the latest report from the European Rapid Alert System for Food and Feed (RASFF 2020-2021) there were 400 notifications for mycotoxin alerts mostly for aflatoxins in nuts, nut products and seeds but also herbs and spices. Based on the RASFF data, chilli, nutmeg, and paprika powder have been the most problematic spices in terms of the frequency in which the maximum regulatory levels were exceeded.



Mycotoxin testing methods

Testing of mycotoxins can be conveniently split into screening methods, where samples are tested to determine whether any mycotoxins are present and quantification methods, where the concentration of mycotoxins present is accurately determined. As analytical technology becomes more advanced the line between these method classes is becoming increasingly blurred.

The methods used for screening of materials for mycotoxins are generally in the group of 'rapid testing' methods, which can provide a result within 30 minutes of the sample being taken [19]. Screening methods are often qualitative or semi-quantitative since they are designed to be used in the field by staff who may not have extensive laboratory training.

In general, these methods provide good sensitivity, however there are obvious challenges in the interpretation of semi-quantitative methods when the concentration in the sample is close to the limit of detection for the method.

The most popular rapid tests are techniques utilising membrane-based immunoassay.

Flowthrough assays are typically based on direct competitive ELISA. The surface of the membrane is coated with the anti-mycotoxin antibody, to which an extract of the sample and a mycotoxin-enzyme conjugate are added. The mycotoxin in the sample extract and the mycotoxin-enzyme conjugate competes for the limited number of antibody binding sites An enzyme substrate is then added which reacts with the mycotoxin coupled enzyme developing a colour. A positive sample, where the mycotoxin from the test material has occupied the antibody sites, will have no coloured spot on the membrane, whereas a negative sample will have a visible spot.

Lateral flow tests are a form of immunochromatography test and are becoming widely used due to their user-friendly protocols and kit stability over time and over a range of conditions. The extracted sample is added to the sample pad in the test kit, any mycotoxin present binds to an anti-mycotoxin antibody gold particle, which together with an anti-2nd antibody gold particle migrates along the membrane. The membranes have two zones, a test zone which has a bound mycotoxin-protein conjugate and a control zone which has a bound mycotoxin-protein conjugate and a control zone which has a bound 2nd antibody. As the chromatographic process draws the sample through these zones, the mycotoxin in the test zone will bind any free anti-mycotoxin antibody gold particle forming visible line. In a positive sample the anti-mycotoxin antibody gold particle will be bound to the mycotoxin present and will not form a coloured line. The completion of the test and confirmation that the chromatography has been sufficient, is seen when the anti-2nd antibody gold particle is captured by the 2nd antibody in the control zone producing a visible coloured line.

> "As analytical technology becomes more advanced the line between these method classes is becoming increasingly blurred..."

Quantitative methods

ELISA (enzyme-linked immunosorbent assay) methods for mycotoxin assay have been available for more than a decade. As with the other immunoassay techniques, the general principle of the technique is the ability of a specific antibody to distinguish the three-dimensional structure of a specific mycotoxin.

The direct competitive ELISA is commonly used in mycotoxin analysis [19],using a conventional microtiter plat, relying on the equilibrium of the antibody–antigen reaction, which would typically require an incubation time of approximately 1–2 h. Currently, most of the commercially available ELISA test kits for mycotoxins are working in the kinetics phase of antibody–antigen binding, which reduces the incubation time to minutes.

After a mycotoxin is extracted from a ground sample with solvent, a portion of the sample extract and a conjugate of an enzyme coupled mycotoxin are mixed and then added to the antibody-coated microtiter wells. Mycotoxin present in the sample extract or control standards is allowed to compete with the enzyme-conjugated mycotoxin for the antibody binding sites. After washing, an enzyme substrate is added to the microtiter wells and a blue colour develops, which is inversely proportional to the concentration of mycotoxin in the sample or standard. A solution is then added to stop the enzyme reaction.

ELISA test kits are widely used as high throughput assays, due to low sample volume requirements and often less sample extract clean-up procedures compared to conventional methods such as HPLC. The methods are rapid, simple, specific, sensitive, portable for use in the field and can be fully quantitative.

As the target compounds are the mycotoxins themselves, compounds with similar chemical groups can also interact with the antibodies, resulting in a matrix effect or matrix interference. The occurrence of a matrix effect in ELISA methods results in underestimates or overestimates in the concentrations measured in commodity samples. Therefore, full validation of an ELISA method for the intended range of commodities is essential and critical.

Chromatography

Although in general the chromatographic assays are costly, time-consuming and require expensive equipment, continual improvements in processing times and instrument capability mean that they are extensively used.

Pre-treatment

Currently, solid phase extraction (SPE) is by far the most popular tech-nique used in the routine analysis of mycotoxins [20], though meth-ods have been reported using liquid-liquid extraction,

supercritical fluid extraction and solid phase micro-extraction (SPME). SPE is a technique based on the use of small chromatography columns, to remove co-extracted contaminants from the test sample, prior to eluting the analytes of interest. A number of alternative stationary phases can be applied for SPE clean-up, depending on both the class of mycotoxins under analysis and the sample matrix. Common cartridges for SPE include, reversed phase C18, silica gel, anion exchange and immunoaffinity columns.

Separation methods

The most widely reported separation method for the analysis of mycotoxins is HPLC, which use various stationary phases, according to the mycotoxin of interest and its physical and chemical structure.

In various field of analysis HPLC-Fluorescence (HPLC-FD) is widely accepted as an official method for the determination of mycotoxins. Several of the mycotoxins have natural fluorescence (ochratoxin A, aflatoxin and citrinin), though many of the other routinely determined compounds (fumonisins) require derivatisation, where o-phthaldialdehyde (OPA) or 9-(fluorenylmethyl)chloroformate (Fmoc) are commonly used, either pre- or post-column.

UPLC/HPLC-MS/MS

More recently, the use of HPLC or UPLC combined with mass spectrometry (MS and MS/MS) detection methods has allowed an increase in sensitivity compared to the older HPLC-FD methods and provided a means of true 'multi-residue' testing, covering a number of the mycotoxin classes in a single analytical run. Researchers have developed analytical methods which can detect between 11 [21] and 33 [22] mycotoxins, simultaneously at sub µg/kg concentrations in food products and animal feed-ing stuffs. Methods using MS/MS are relatively robust meaning that simplified, time-efficient, sample clean-up protocols can be applied, potentially leading, over time, to the possibility of their application for rapid sample screening.

Findings from Proficiency Testing (PT)

The widespread testing of a wide range of commodities for the presence of mycotoxins, to multiple, stringent, regulatory limits, means that there is a high requirement for extensive quality control for these methods. Proficiency Testing (PT) is a vital component of the quality control toolbox and forms a key part of the accreditation of test laboratories to the ISO/IEC 17025 standard.

A recent round of PT within the AXIO QFCS scheme was based on the analysis of mycotoxins in rice. A test material was prepared and distributed to participants, which contained aflatoxins (AFB1, AFB2, AFG1 and AFG2), Ochratoxin A (OTA) and Zearalenone (ZON).

Proficiency testing requires the participants to use their routine testing methods to determine the concentrations of the analytes of interest.

Across all of test analytes the most common method used by the participants in the PT scheme was LC-MS/MS, which accounted for ~50% of the total number of results for each of the individual mycotoxins tested. The only significant difference was observed for Total Aflatoxins where there was an increase in the proportion of participants reporting results using ELISA methods and corresponding decrease in the proportion of results returned for the chromatography (HPLC-FD and LC-MS/MS) methods.

ELISA data was only returned/available for two of the aflatoxin measurands, AFB1, which is the most potent natural carcinogen and usually the major aflatoxin produced by toxigenic strains, and total aflatoxins. This is presumably because no individual legislative levels exist for AFB2, AFG1 and AFG2.

	AFB1 (µg/kg)	Total AF (µg/kg)	OTA (µg/kg)	ZON (µg/kg)
LC-MS/MS	1.58	3.95	3.96	43.4
HPLC-FD	1.43	3.68	3.38	27.3
ELISA	0.71	2.30	1.80	48.5
Other	1.60	2.73	2.37	87.4
All Methods	1.45	3.25	3.11	35.4

The average values of three of the four analytes showed higher results for the HPLC-FD/LC-MS/MS methods compared to the ELISA method. The methodological differences were small however, relative to the acceptability criteria for the PT so an assigned value based on all of the data returned could be used.

	AFB1	AFB2	AFG1	AFG2	AF Tot.	OTA	ZON
AV (µg/kg)	1.45	0.38	1.64	0.43	3.25	3.11	35.4
% Satisfactory results	73.9	81.3	87.5	86.7	75.0	58.6	69.6
% Questionable results	4.3	6.3	12.5	6.7	12.5	13.8	4.3
% Unsatisfactory results	21.7	12.5	0.0	6.7	12.5	27.6	26.1

The performance of the participants in the PT round were typically good with ~70% or more of participants receiving a satisfactory performance assessment. The rate of satisfactory performance was higher for AFB2, AFG1 and AFG2, for which only the HPLC and LC-MS/MS methods were used. A num-ber of the unsatisfactory performance assessments were for results which were considered outliers, and which were not included in the calculation of summary statistics. Common reasons for such large errors are non-analytical errors, such as transcription, unit or calculation errors, or analytical errors such as incorrect dilutions.

Conclusion

Spices are globally important products, which are predominantly used to improve the flavour and appearance of many of the most popular food products.

Although they are used in relatively small quantities, the quality and safety of spices is paramount to the overall safety of foods.

Spices may contain a number of different contaminants, some of which are microbiological and other which are chemical in nature. One of the most frequently detected contaminants of spices is the group of chemicals identified as mycotoxins.

A by-product of microbial growth, mycotoxins are persistent and can have serious consequences for the health of humans and other vertebrates.

Spices, therefore, are routinely tested for the presence of microorganisms and mycotoxins. The performance of labs undertaking such testing can be assessed by proficiency testing, a third party quality control tool.

AXIO PT schemes have demonstrated the good performance of labs undertaking microbiological and mycotoxin testing over a number of years. The detailed reports provided, offer a range of benefits for participant laboratories, including the comparison of labs with their peers, evaluation of performance at legislative levels and the comparison of analytical methods.

The Authors

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Tracey Noblett is Head of Microbiology for the Proficiency Testing group at LGC AXIO. She is responsible for the production, development and technical support for a number of microbiology proficiency testing schemes covering the water, environment, food, animal feeds, pharmaceuticals and consumer safety industries. Prior to joining LGC in 2006, Tracey gained 20 years' microbiology experience in a variety of fields, including clinical, water and food. Since 2008 she has held the position of Secretariat for the Eurachem Proficiency Testing Working Group.

Savvas Xystouris

Savvas is the Technical Development Manager for Chemistry LGC AXIO proficiency testing at LGC. He is mainly responsible for the development of new proficiency testing materials and technical management of PT schemes. He has a background in Chemistry and Food Science and Nutrition. Prior to joining LGC, he has worked at the State General Laboratory (SGL) of Cyprus as an Analytical Chemist. Savvas has been involved in the European Food Safety Authority's (EFSA) FoodEX II project, for the classification and re-coding of various food products as part of SGL's EFSA Focal point projects. He is also member of the Institute of Food Science and Technology (IFST) since 2011.

Spice PT samples by AXIO

PT-MC-06SP *Salmonella* in spices

PT-MC-24SP Lactic acid bacteria in spices

PT-MC-31 The detection of *Salmonella* species in spices

PT-MC-36SP Quantitative package in spices

PT-FC-794 Aflatoxin analysis in spices

PT-FC-815 Chemical parameters of spices

PT-FC-825 Authenticity of herbs and spices

PT-FC-843 Pesticides in spices

PT-FC-868 Ethylene oxide in spices

PT-FC-869 Water activity in spices

PT-FC-870 Bulk index of spices

Mycotoxin PT samples by AXIO

PT-FC-779 Aflatoxin analysis in nuts

PT-FC-794 Aflatoxin analysis in spice

PT-MP-03 Mycotoxin analysis in malt flour

PT-AF-05 Mycotoxin analysis in animal feed

PT-BV-520 Patulin in soft drinks

PT-CH-60 Aflatoxin M1 in milk

PT-CH-62 Aflatoxin M1 in soft cheese

PT-FC-804 Mycotoxins in dried fruits

PT-CA-03 Mycotoxins in cannabis

PT-FC-845 Mycotoxins in rice

PT-FC-867 Mycotoxins in corn

Have a question about one of our PT samples?

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Bibliography

[1] Introduction. Gernot Katzer's Spice Pages. http://gernot-katzers-spice-pages.com/engl/spice_intro.html Retrieved 28 February 2021.[

[2] Angelon-Gaetz KA, Klaus C, Chaudhry EA, Bean DK. Lead in Spices, Herbal Remedies, and Ceremonial Powders Sampled from Home Investigations for Children with Elevated Blood Lead Levels North Carolina, 2011–2018. MMWR Morb Mortal Wkly Rep 2018;67:1290–1294. DOI: http://dx.doi.org/10.15585/mmwr.mm6746a2external icon.

[3] https://www.ua-bw.de/pesticides/beitrag_en.asp?subid=1&Thema_ID=5&ID=3129&Pd-=No&lang=EN#:~:text=The%20five%20most%20frequently%20occurring,the%20legally%20established%20maximum%20level.

[4] https://food.ec.europa.eu/system/files/2021-11/food-fraud_action_herbs-spices_report_jrc126785_0.pdf [5] https://www.food.gov.uk/sites/default/files/media/document/pahs-in-herbs-spices-supplements- and-tea-final-report.pdf

[6] https://www.ifst.org/resources/information-statements/mycotoxins

[7] Microbial profile of common spices and spice blends used in Tamale, Ghana. Food Contamination 4, 10 (2017). Bakobie, N., Addae, A.S., Duwiejuah, A.B. et al https://doi.org/10.1186/s40550-017-0055-9

[8] ACM/913 ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD INFORMATION PAPER 2004 - Microbiological Examination of Dried Spices and Herbs from Production and Retail Premises in the United Kingdom https://acmsf.food.gov.uk/sites/default/files/mnt/drupal_data/sources/files/multimedia/pdfs/committee/ acm913spices.pdf

[9] Spice-related outbreaks in the U.S https://www.healthline.com/health-news/food-contaminated-spices-found-amid-food-imports-082813#Spice-Related-Outbreaks-in-the-U.S.

[10] Processes to Preserve Spice and Herb Quality and Sensory Integrity During Pathogen Inactivation Susan E. Duncan, Kayla Moberg, Kemia N. Amin, Melissa Wright, Jordan J. Newkirk, Monica A. Ponder, Gary R. Acuff, James S. Dickson J Journal of Food Sci. 2017 May; 82(5): 1208–1215. Published online 2017 Apr 13. doi: 10.1111/1750-3841.1370 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5435955/

[11] https://www.frontiersin.org/articles/10.3389/fmicb.2019.02602/full#B31

[12] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2016.4654

[13] https://www.efsa.europa.eu/en/efsajournal/pub/4902

[14] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6356975/

[15] CODE OF PRACTICE FOR THE PREVENTION AND REDUCTION OF MYCOTOXINS IN SPICES CXC 78-2017

[16] https://www.fao.org/fao-who-codexalimentarius/committees/committee/related-standards/en/?committee=CCSCH

[17] https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252F-workspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B193-1995%252FCXS_193e.pdf

[18] https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252F-workspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-735-12%252FWD%252Fcf12_11e.pdf

[19] Zheng, M. Z., Richard, J. L., Binder, J. 2006 A review of rapid methods for the analysis of mycotoxins Mycopathologia 161: 261–273

[20] Turner, N. W., Subrahmanyam, S., Piletsky, S. A. 2009 Analytical methods for determination of myco- toxins: A review. Analytica Chimica. Acta 632. 168–180

[21] Sameni, M., Dubecke, A., Weber, J-F. F. 2014 Simultaneous multi-residue determination of mycotoxins in foods using LC-MS/MS. J Environ Anal Toxicol. 5:2

[22] Stead, S., Roberts, D., Gledhill, A., de Rijk, T., Van Poucke, C., De Saeger, S. 2014 The Development of a Sensitive Multi-Residue LC-MS/MS Method for the Quantitative Determination of Mycotoxins in Animal Feeduffs and Silage Using Xevo TQ-S, Waters Application Note

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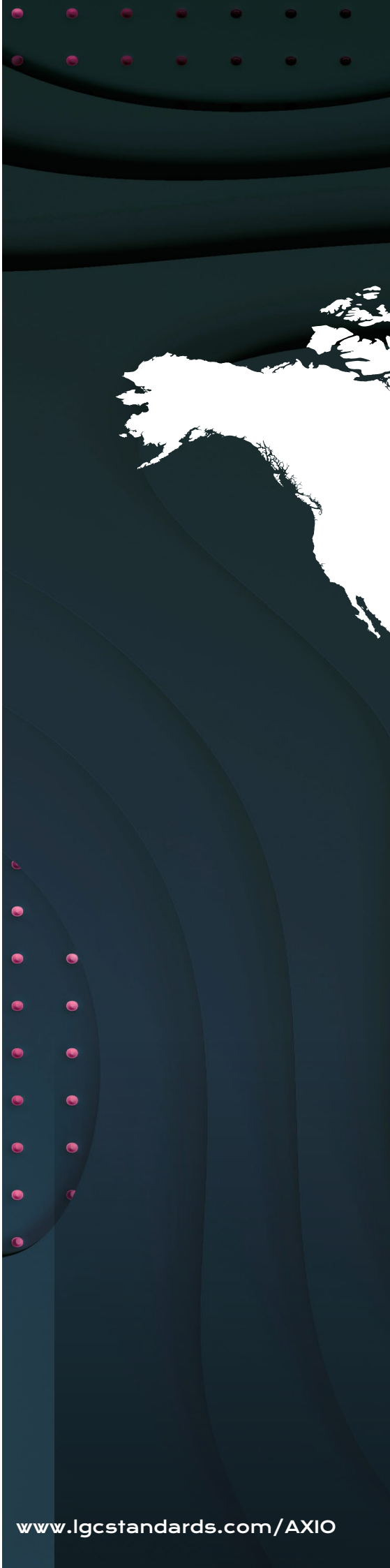

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