Overview and latest advances in fumonisins

Gordon S. Shephard

Introduction

The fumonisin mycotoxins are an important group of secondary fungal metabolites relatively recently discovered that have elicited intense international interest and concern due to their carcinogenic nature and ubiquitous occurrence in maize, a major world grain crop and staple diet of many people in the developing world. The fumonisins are primarily produced by Fusarium verticillioides (Sacc.) Nirenberg (=F. verticillioides)
such as N-carboxymethyl-FB and N-(1-deoxy-D-fructos-1-are known, as well as certain reaction products with sugars, these forms, a number of partially and fully hydrolysed forms including members of the A,B, C and P series [39]. Besides tricarballylic acid [4]. Although the published literature on groups are esterified with the terminal carboxyl group of (FB ), B (FB ) fumonisins are those of the B series, namely fumonisins Klittich, Leslie, Nelson et Marasas [39]. The most important napiforme major pathogens of maize (Zea mays L.) around the world [30]. The only other Fusarium species thus far shown to be a high producer of fumonisins is F. nygamai Burgess et Trimboli. Several other species are moderate to low producers, some of which include F. anthophilum (A. Braun) Wollenw., F. globosum Rheeder, Marasas et Nelson, F. napiforme Marasas, Nelson et Rabie and F. thapsinum Klittich, Leslie, Nelson et Marasas [39]. The most important F. globosum species thus far shown to be a high producer of fumonisins is F. verticillioides and B (FB ) (Figure 1). These compounds are diesters of propane-1,2,3-tricarboxylic acid (tricarballylic acid) and various 2-amino-1,2,6-dimethyl-polycarboxylic acids in which the C-14 and C-15 hydroxy groups are esterified with the terminal carboxyl group of tricarballylic acid [4]. Although the published literature on fumonisins deals almost exclusively with these three forms, a recent review listed a total of 28 fumonisin analogues, including members of the A,B, C and P series [39]. Besides these forms, a number of partially and fully hydrolysed forms are known, as well as certain reaction products with sugars, such as N-carboxymethyl-FB, and N-(1-deoxy-D-fructos-1-yl)FB, [21, 36, 46, 64]. Evidence has also been presented for the combination of fumonisins with proteins in processed foods [27]. However, apart from the three main toxins of the B series, the extent of the natural occurrence and the significance of many of these forms are largely unknown.

Toxicology
The discovery of the fumonisin mycotoxins was immediately followed by experimental evidence proving them to be the causative agents for two animal diseases previously associated with the ingestion of F. verticillioides-contaminated feed. In 1990, Kellerman et al. [26] reproduced the brain lesions associated with equine leukoencephalomalacia by oral dosing of pure FB to horses, and Harrison et al. [20] reproduced pulmonary oedema and hydrothorax in swine by intravenous dosing of FB. The effects of fumonisins on animal species vary and have been recently summarized [6]. Apart from an association with an outbreak of food-borne disease characterized by abdominal pain and diarrhoea reported by persons consuming fumonisins-contaminated maize and sorghum on the Deccan Plateau in India [5], direct human toxicity has not been reported. Concern over the effects on human health due to exposure to these mycotoxins arises from their carcinogenic properties. FB is both a cancer initiator and promoter and has been shown to be hepatocarcinogenic in male BDIX rats at a level in feed of 50 mg/kg [19]. The carcinogenic properties of FB have been confirmed by the National Toxicology Programme study in the USA which demonstrated FB to be nephrocarcinogenic in male F344/N rats and hepatocarcinogenic in female B6C3F1 mice, both at 50 mg/kg feed [22]. The fumonisins have been associated with the high incidence of oesophageal cancer in the Transkei region of South Africa [38] and in Luxin county, Henan Province and Cixian county, Hebei Province in China [9, 70], as well as the incidence of liver cancer in the Haimen region, Jiangsu Province of China [60]. More recent concerns over human exposure to fumonisins mycotoxins relate to their possible role in the incidence of neural tube defects (NTDs). FB has been shown to inhibit folic acid uptake by the folate receptor in vitro, an effect mediated by the cellular decrease in sphingolipid levels [52]. Experimental evidence for this role has recently been published and it shows that FB can cause cranial NTDs in whole mouse embryo culture and that these effects can be ameliorated by folic acid [43].

Natural occurrences
F. verticillioides and F. proliferatum occur widely in maize, the primary sources of animal and human exposure to fumonisins are maize and maize-based products. Reviews of published data on natural occurrence list over 35 countries in which fumonisins have been detected in maize [6, 50]. Although a wide variation in levels have been found, the highest level ever reported was found in US maize screenings used for animal feed (330 mg/kg) [41], while exceptionally high levels have also been found in home-grown maize in Transkei region (117 mg/kg) [38] and in Luxin county (155 mg/kg) [9]. The fumonisins occur mainly in the hull (pericarp) and germ of the kernel, and least in the starch. As such, the fumonisins can be variously distributed in commercially milled products by the type and nature of the milling process that the maize kernels undergo. During the wet milling process, fumonisins can be partly eliminated in the steep water and occur at decreasing levels in the resultant fractions of gluten, fibre, germ and starch, respectively [3]. As a result of dry milling, fumonisins occur at decreasing levels in bran, germ, flour and flaking grits [25]. Fumonisins do not appear to be present in oil, sweeteners or ethanol produced from maize. Apart from maize, other commodities that have been found to be contaminated include rice, sorghum, beans, beer and asparagus [6]. A particular concern is the co-occurrence of fumonisins and aflatoxins. This has been reported from a number of countries, including Brazil [34, 62], China [60], Guatemala [59], India [5, 51], Indonesia [1, 69], Philippines [69], Thailand [69], USA [8], Venezuela [31] and Vietnam [65].

Regulations and evaluations
The recent FAO survey of worldwide mycotoxin regulations lists 6 countries (Bulgaria, Cuba, France, Iran, Switzerland and USA) with legislated or recommended maximum tolerated levels [17]. In 1993, the International Agency for Research on Cancer (IARC) declared the toxins produced by Fusarium moniliforme to be possibly carcinogenic to humans (group 2B carcinogens) [61]. Subsequently, this assessment has been refined and FB itself was evaluated as a group 2B carcinogen [23]. The Scientific Committee on Food of the European Commission evaluated toxicity and carcinogenicity studies on FB, and has recommended a “tolerable daily intake (TDI)” of 2 μg/kg body weight/day based on a no observed adverse effect level (NOAEL) in rodent studies and a safety factor of 100 [14]. Based on similar considerations, the recent 56th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) determined a group provisional maximum tolerable daily intake (PMTDI) for FB, FB, and FB, alone or in combination,
of 2 μg/kg body weight/day [6]. Following this decision, the Scientific Committee on Food of the European Commission updated its own fumonisin evaluation and similarly established a group of TDI for FB₁, FB₂ and FB₃, alone or in combination, at 2 μg/kg/day [15].

Analytical methods

Sampling

As in all mycotoxin analytical work, the final result needs to be understood in terms of the variances associated with all the steps of the analytical process including sampling, sample preparation and the actual laboratory determination. In a study on shelled maize lots, it was found that relative to aflatoxin, fumonisins are more evenly distributed within a lot [67]. In determining the total variance associated with the process, 1.1 kg samples were withdrawn from the lots and 25 g sub-samples were subjected to a single high-performance liquid chromatography (HPLC) determination. At a contamination level of 2 μg/kg, sampling accounted for 61.0% of the total variance, sample preparation, 18.2% and sample analysis, 20.8%. Based on a compound gamma distribution, an operating characteristics curve can be generated for fumonisins which defines the producer and consumer risk profiles for a given fumonisin acceptance level [66].

Extraction

The fumonisins are polar compounds which are generally extracted from maize by shaking (30-60 min) or blending (3-5 min) using either acetonitrile/water (1:1) or methanol/water mixtures (70-80% methanol) [2, 13, 40, 49, 54, 56, 57]. Other authors have suggested improvements can be achieved using acidified extraction solvents [71]. In contrast, recent work on rapid methods of analysis such as fluorescence polarization have employed purely aqueous solvents [29]. Various solvent mixtures (70-80% methanol) [2, 13, 40, 49, 54, 56, 57]. Other authors have suggested improvements can be achieved using acidified extraction solvents [71]. In contrast, recent work on rapid methods of analysis such as fluorescence polarization have employed purely aqueous solvents [29]. Various solvent mixtures (70-80% methanol) [2, 13, 40, 49, 54, 56, 57]. Other authors have suggested improvements can be achieved using acidified extraction solvents [71].

Clean-up

Sample extracts can be used directly for determination of total fumonisins (as in enzyme-linked immunosorbent assays, ELISA) or need to be cleaned-up and concentrated prior to chromatographic separation and determination of individual fumonisins. All clean-up methods for determination of fumonisins are based on solid phase extraction using different chemical or immunoaffinity principles. In all cases, the properties of the filtered extract need to be modified to suit the requirements of the individual methods. Immunoaffinity columns, which require aqueous dilution of the extract, achieve the most effective clean-up and are specific and efficient solvent, are usually relatively expensive [11, 13, 63]. Conditions appropriate for the re-use of these columns have been investigated [18]. Adequate clean-up of maize extracts can be achieved with strong anion exchange (SAX) cartridges as well provided the extract pH is above 5.8 [54, 56], while reversed-phase (C₈) cartridges can be used provided the organic content of the extract is reduced by aqueous dilution [2, 40]. For determination of both fumonisins and their hydrolysed moieties, combinations of SAX and (C₈) have been employed [45].

Chromatographic Separation

Generally, these polar mycotoxins are ideally suited for reversed-phase HPLC separation. The fumonisins lack a suitable UV chromophore for HPLC detection and hence most routine analytical methods rely on precolumn derivatisation with a fluorescent tag such as o-phthalaldehyde (OPA) and separation of the resultant derivatives [49, 50, 54, 56]. The method can be automated [12], while a suitable post-column derivatisation method has also been published [32]. Evaporative light scattering detection of underivatised fumonisins has been used for the estimation of the purity of fumonisin standards [68]. The coupling of liquid chromatography and mass spectrometry (LC-MS) by atmospheric pressure ionization (API) techniques, especially the development of the electrospray interface, has enabled highly sensitive and specific fumonisin methods to be developed [10, 24]. The use of LC-MS/MS can provide both quantification of fumonisins and confirmation of their presence based on the production of a spectrum of highly specific fragment ions from the protonated molecular ion.

Despite the development of sophisticated techniques such as LC-MS, thin layer chromatography (TLC) remains an important technique in many laboratories, especially in the developing world. The first TLC method was developed on silica plates using p-anisaldehyde spray reagent and was employed in the original isolation of the toxins [7]. Use of this method for quantification was restricted by its limit of detection (500 μg/kg). The use of reversed-phase TLC separation of fumonisins, followed by fluorescamine spray for visualization, improved the detection limit to below 1 μg/kg and allowed the method to be used for naturally contaminated maize [42]. The detection limit of the original method, which used reversed-phase (C₈) solid phase extraction clean-up, was improved by the use of SAX cartridges [33]. Further improvements in the TLC detection limits of this method to 0.1 μg/kg in maize have been achieved by using immunoaaffinity column clean-up and scanning fluorodensitometry [37]. A recent publication reported the development and collaborative study of a TLC method based on prederivatization before TLC separation [48].

Total Fumonisins

In contrast to the chromatographic methods for the determination of the individual fumonisin analogues, a number of methods have been developed for rapid total fumonisin determination which are based on the immunological recognition of a fumonisin epitope. As in the case of a number of other mycotoxins, ELISA methods have been developed and commercialized based on both polyclonal and monoclonal antibodies [35, 55]. The use of ELISA techniques represents a convenient and rapid method for high throughput screening of maize samples. However, their use should be restricted to the levels of fumonisin contamination and to the matrices recommended by the manufacturers. Although immunoaaffinity columns are mostly applied for clean-up of sample extracts prior to HPLC, their use for the preparation of a purified sample suitable for total fumonisin determination by fluorescence derivatization and direct measurement, has also been commercialized [13]. Fluorescence polarization is another rapid method for fumonisin determination that relies on fumonisin antibodies and the competition for binding sites between the unknown fumonisin of a sample and a suitable fluorescent fumonisin derivative [29]. The development of biosensor technology is an area of current interest for mycotoxin analysis. The basic concept of biosensor techniques is the measurement of a physical or chemical change that occurs on the surface of the sensor due to the
Due to the worldwide interest and concern over food safety, the determination of fumonisins will increasingly become a routine requirement in food laboratories. The current HPLC and immunassay methods are well suited for analysis at all regulatory levels, while TLC methods are also available and biosensors offer the promise of more rapid methods in the future. However, the issue of fumonisin extraction from processed foods, the presence of reaction products with sugars and possibly “hidden” fumonisins linked to proteins still need to be investigated.

References
9. Elkins, J., S.C. Bialik, T.G. Robinowich, J.B. Crouse, 1989. Fluorescent isothiocyanate conjugate has been developed and favourably compared with HPLC results [28, 58].


