# Overview and latest advances in fumonisins

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**Revisión general y avances recientes en fumonisinas**

**Resumen**. Desde que fueron identificadas por primera vez en 1988, las fumonisinas han llamado considerablemente la atención debido a sus propiedades cancerígenas y a su ocurrencia casi universal en el maíz y en los productos fabricados a base de maíz. Esta revisión, basada en una presentación del Primer Simposio Panamericano de Micotoxinas para la Industria, tratará brevemente sobre su producción, ocurrencia natural, toxicología y de su valoración internacional reciente. Se presenta el estatus actual de los métodos disponibles para su determinación analítica, incluyendo extracción, purificación y etapas de separación cromatográfica, así como los métodos más rápidos para la determinación de fumonisinas totales basados en el reconocimiento inmunológico. Se revisan los métodos disponibles para la determinación de fumonisinas incluidos los métodos oficiales basados en la cromatografía líquida de alta resolución (HPLC), separación de derivados fluorescentes, métodos de escreening basados en la cromatografía de capa fina (TLC) y métodos avanzados de investigación basados en la cromatografía líquida acoplada a masas (LC-MS).
**Palabras clave**: Fumonisinas, micotoxinas, alimentos, maíz, *Fusarium verticillioides*.

**Abstract.** Since they were first identified in 1988, the fumonisins have attracted considerable international attention due to their carcinogenic properties and almost universal natural occurrence in maize and maize-based products. This review, based on a presentation to the First Pan-American Symposium on Mycotoxins for Industry, will briefly deal with their fungal production, natural occurrence, toxicology and recent international assessment. The current status of methods available for their analytical determination, including extraction, clean-up and chromatographic separation steps will be highlighted, as well as the more rapid methods for the determination of total fumonisin based on immunological recognition. Chromatographic methods currently available for the determination of fumonisins include official methods based on high-performance liquid chromatography (HPLC) separation of fluorescent derivatives, screening methods based on thin layer chromatography (TLC) and advanced research methods based on liquid chromatography-mass spectrometry (LC-MS).
**Keywords:** fumonisins; mycotoxins; food; maize; *Fusarium verticillioides*

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## 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.*

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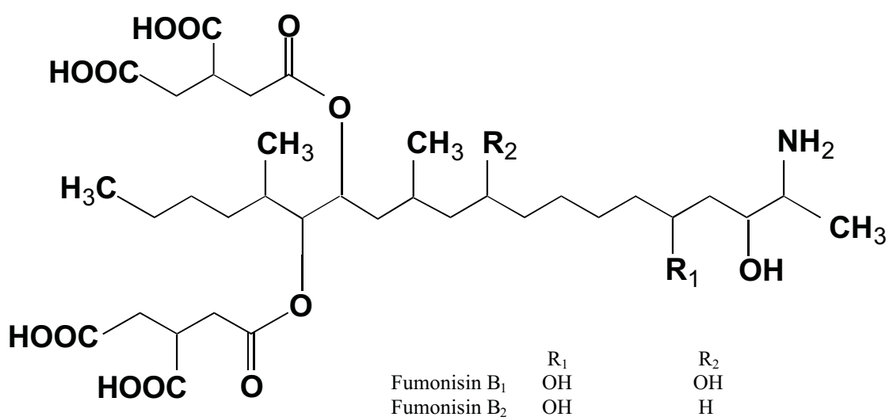
*moniliforme* Sheldon) and *F. proliferatum* (Matsushima) Nirenberg, major pathogens of maize (*Zea mays* L.) around the world [30]. The only other *Fusarium* species thus far shown to be a high producer of fumonisins is *F. nygamai* Burgess et Trimboli. Several other species are moderate to low producers, 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 fumonisins are those of the B series, namely fumonisins $B_1$ ($FB_1$), $B_2$ ($FB_2$) and $B_3$ ($FB_3$) (Figure 1). These compounds are diesters of propane-1,2,3-tricarboxylic acid (tricarballylic acid) and various 2-amino-12,16-dimethyl-polyhydroxyeicosanes 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-$FB_1$ and N-(1-deoxy-D-fructos-1-yl)$FB_1$ [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 $FB_1$ to horses, and Harrison *et al*. [20] reproduced pulmonary oedema and hydrothorax in swine by intravenous dosing of $FB_1$. 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 fumonisin-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. $FB_1$ 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 $FB_1$ have been confirmed by the National Toxicology Programme study in the USA which demonstrated $FB_1$ to be nephrocarcinogenic in male F344/N rats and hepatocarcinogenic in female B6C3$F_1$ 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 Linxian 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 fumonisin mycotoxins relate to their possible role in the incidence of neural tube defects (NTDs). $Fb_1$ 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 $FB_1$ can cause cranial NTDs in whole mouse embryo culture and that these effects can be ameliorated by folic acid [43].




| | $R_1$ | $R_2$ |
|---|---|---|
| Fumonisin $B_1$ | OH | OH |
| Fumonisin $B_2$ | OH | H |
| Fumonisin $B_3$ | H | OH |

Figure 1. Chemical structure of fumonisins $B_1$, $B_2$ and $B_3$.

**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 Linxian 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 $FB_1$ itself was evaluated as a group 2B carcinogen [23]. The Scientific Committee on Food of the European Commission evaluated toxicity and carcinogenicity studies on $FB_1$ 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 $FB_1$, $Fb_2$ and $Fb_3$, 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 $FB_1$ , $Fb_2$ and $Fb_3$, 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 subsamples were subjected to a single high-performance liquid chromatography (HPLC) determination. At a contamination level of 2 mg/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 to sample ratios, ranging between 2:1 and 10:1 have been used by analysts. Methods of analysis based on extraction with aqueous organic mixtures generally achieve analytical recoveries from maize within the guidelines laid down by the European Committee for Standardization (1999) [16]. However, in maize-based processed foods such as maize bran flour, baby foods and breakfast cereals, poor recoveries have been experienced [44, 47]. These problems were originally highlighted by Scott and Lawrence (1994) [44], who investigated acidified and alkaline solvents for extraction of fumonisins from maize bran flour and breakfast cereals. A recently published official method to determine fumonisins in corn flakes employed a double extraction with acetonitrile/methanol/water (1:1:2) [63], while a study on Brazilian baby foods indicated acidified aqueous methanol to be the best overall solvent for a range of such foods, but not necessarily the best for each individual type of food [47].

**Clean-up**

Sample extracts can be used directly for determination of total fumonisins (as in enzyme-linked immunoabsorbent 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 solvent efficient, but are 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 ($C_{18}$) 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 ($C_{18}$) 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-phthaldialdehyde (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 mg/kg). The use of reversed-phase TLC separation of fumonisins, followed by fluorescamine spray for visualization, improved the detection limit to below 1 mg/kg and allowed the method to be used for naturally contaminated maize [42]. The detection limit of the original method, which used reversed-phase ($C_{18}$) solid phase extraction clean-up, was improved by the use of SAX cartridges [53]. Further improvements in the TLC detection limits of this method to 0.1 mg/kg in maize have been achieved by using immunoaffinity 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 poly- 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 immunoaffinity 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

binding of the analyte to a receptor (eg. antibody) immobilized on that surface. Typically, evanescent wave techniques such as surface plasmon resonance [33] or fluorescence coupling on an optical fibre [58] can be used and both these techniques have been applied to fumonsin measurement. A fibre-optic immunosensor, using a monoclonal antibody as receptor and a $FB_1$-fluorescein isothiocyanate conjugate has been developed and favourably compared with HPLC results [28, 58].

## Conclusions

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 immunoassay techniques are well suited for analysis at possible 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 possible "hidden" fumonisins linked to proteins still need to be investigated.

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