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ANALYSIS OF FUMONISINS: A REVIEW

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ABSTRACT

Fumonisins are produced mainly by *Fusarium* species and have an adverse effect on human and animal health. To quantify and qualify fumonisins in foods and feeding stuffs, several methods have been developed such as enzyme-linked immunosorbent assay (ELISA), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography - mass spectrometry (LC-MS) and gas chromatography - mass spectrometry (GC-MS) techniques. Most of the methods are applied to quantify fumonisin Bs because of their dominant presence among fumonisin analogs. In this review, the principles of the methods are discussed and their advantages and limitations are analyzed as well.

Keywords: Analysis, chromatographic methods, ELISA, fumonisin.

Phân tích độc tố nấm mốc Fumonisin: Bài tổng hợp

TÓM TẮT

Độc tố nấm mốc fumonisin được tạo ra chủ yếu bởi những loài nấm Fusarium và gây ảnh hưởng nghiêm trọng đến sức khỏe của động vật và người. Để phân tích định lượng và định tính fumonisin trong thức ăn và nguyên liệu sản xuất thức ăn, nhiều phương pháp đã được áp dụng như ELISA, sắc ký lớp mỏng (TLC), sắc ký hiệu năng cao (HPLC), sắc ký lỏng ghép đầu dò khối phổ (LC-MS) và sắc ký khí ghép đầu dò khối phổ (GC-MS). Hầu hết các phương pháp đều áp dụng để phân tích định lượng fumonisin nhóm B vì nhóm này xuất hiện nhiều hơn hẳn so với những nhóm khác. Bài tổng hợp này sẽ thảo luận những nguyên lý của phương pháp, đồng thời cũng phân tích những ưu điểm và giới hạn của phương pháp.

Từ khóa: ELISA, fumonisin, phân tích, phương pháp sắc ký.

1. INTRODUCTION

The fumonisins, first isolated by Gelderblom *et al.* (1988), are a group of mycotoxins produced by many *Fusarium* species, mostly by *Fusarium proliferatum* and *Fusarium verticillioides* (former name is *Fusarium moniliforme*). It was believed that fumonisins were only produced by *Fusarium* species until the year of 2000. However, other fungi can also synthesize fumonisin, such as *Aspergillus niger* (Frisvad *et al.*, 2007) and *Aspergillus awamori* (Varga *et al.*, 2010). The presence of fumonisin mycotoxins in foods and feeds is one of the most serious concerns recently because of their harmful effects on animal and human health. The presence of fumonisin B_1 (FB_1) is the most frequent among fumonisins in maize, representing about 60% of total fumonisins (Voss *et al.*, 2011). Fumonisin B_1 is classified in Group 2B, as it may cause cancer in humans (IARC, 1993). Fumonisin intake, in relatively high doses and after a prolonged feeding, has been reported to cause

porcine pulmonary edema (PPE), equine leukoencephalomalacia (ELEM) and liver damage in most species including pigs, horses, cattle, rabbits, and primates, and moreover, kidney damage in rats, rabbits, and sheep (Voss, 2007). To reach the demands of physiological research on the effects of fumonisin intake, there is a continuous development in the field of quantitative analysis of fumonisins. This review is to give an overview and a comparison of these assays.

2. CHEMICAL STRUCTURE OF FUMONISINS

Four groups of fumonisins (FA, FB, FC and FP) were classified based on structure of their backbone and that of the functional groups at positions C1, 2, 3 and 10. (Musser & Plattner, 1997). The fumonisin B group is the most abundant among fumonisins produced by fungal species. Theoretically, there are thousands of isomers of fumonisins those can be synthesized based on chiral centers of fumonisin structure (Bartók *et al.*, 2010b). More than 100 isomers and stereoisomers of fumonisins were asserted by researchers (Rheeder *et al.*, 2002; Bartók *et al.*, 2008; Bartók *et al.*, 2010b; Varga *et al.*, 2010). The chemical structure of fumonisins consists of a 19-carbon amino-polyhydroxylalkyl chain (fumonisin C) or a 20-carbon amino-polyhydroxyalkyl chain (fumonisin A, B, P) and some different chemical groups (*N*-acetyl amide, amine, tricarboxylic) depending on the type of fumonisin analogue (Table 1, Figure 1). Basically, compounds at the carbon position number 14 and 15 are *tricarballylic acid* (TCA) and they can be found in all groups of fumonisins except some isomers. Different fumonisin analogs are also distinguished by the interchange of hydrogen and hydroxide in the C-3 and C-10 positions. The highest extent of differences among chemical structures of fumonisins is in the C-2 position. These groups are the *N*-acetyl amide ($NHCOCH_3$) in the fumonisin A group, the amine (NH_2) in fumonisin B and C, and the 3-hydroxypyridinium (3HP) moiety in fumonisin P.

3. EXTRACTION AND PURIFICATION

3.1. Extraction

The selection of the extraction method is based on the type of matrix and the target fumonisin. Fumonisins are soluble in water and polar solvents such as methanol and acetonitrile owing to the presence of carboxyl moieties and hydroxyl groups. According to Tamura *et al.* (2014), FA can be extracted by an aqueous solution of acetic acid mixed with acetonitrile (1:1, v/v). In the case of FC and FP, a mixture of methanol and distilled water (70:30, v/v) and (75:25, v/v) was used, respectively (Lazzaro *et al.*, 2013; Bartók *et al.*, 2014). Water was used successfully in extracting FB_1 and FB_2 from taco shells, corn-based products, and rice (Lawrence *et al.*, 2000). Sewram *et al.* (2003) reported that the most efficient method is using acidified 70% aqueous methanol at pH 4.0 to improve the extraction of fumonisin B_1, B_2 and B_3 from corn-based infant foods. Scott *et al.* (1999) studied the extraction of fumonisins from several sorts of foods and foodstuffs manufactured from rice, corn and beans. Four types of solvent mixtures were used including methanol: acetonitrile: water (25: 25: 50), methanol: water (75: 25 or 80: 20), sodium hydrogen phosphate: acetonitrile (1: 1) and methanol: borate buffer (3: 1). As a result, the combination of methanol:acetonitrile:water (25: 25: 50) proved to be the most efficient extraction solvent mixture for fumonisins.

Besides the composition of the extraction solvent, its temperature can also exert an effect on the performance of the extraction. According to Lawrence *et al.* (2000), when the extraction was accomplished at 80ºC from taco shells, the efficiency of the extraction with methanol:acetonitrile:water mixture (25:25:50) was three times more effective than at 23ºC, while the quantity of fumonisins extracted with ethanol:water (3:7) was approximately doubled when the temperature of the extraction solvent was increased from 23º to 80ºC. Moreover, the ethanol/water extraction was the cheapest and the least toxic among the used methods. Nevertheless,

in the presence of water and at high temperatures, samples with high starch content tend to form gels that can hamper the extraction.

3.2. Purification

The resulting extract is usually purified. Several purification methods have been used including solid phase extraction (SPE) with an octadecyl (C18) stationary phase, strong anion-exchange (SAX) cartridges and immunoaffinity columns (IAC). In order to purify FB_1, extraction using a novel centrifugal partition chromatography (CPC) method was applied (Hübner *et al.*, 2012; Szekeres *et al.*, 2012).

Extraction and purification by SPE using C18 cartridges can be applied for various sorts of mycotoxins including aflatoxin, fumonisin, deoxynivalenol, ochratoxin A, T-2 toxins and zearalenone (Romero-Gonzalez *et al.*, 2009). Reversed-phase SPE using C18-type stationary phases has been reported also as an applicable tool to extract and purify samples when fumonisins and their hydrolyzed metabolites are to be analyzed (Poling & Plattner, 1999; Mateo *et al.*, 2002).

SAX-cartridges are highly effective in purification of the extracts. However, SAX cannot be applied to purify the hydrolyzed derivatives of FBs because of the lack of the carboxylic group (Shephard, 1998). SAX was reported to be an appropriate method to extract fumonisins from untreated maize but proved to be ineffective for products with high fat content such as maize based snack products or cornflakes (Meister, 1999).

IAC clean-up is another choice of sample purification. Like the SAX method, IAC cannot retain hydrolysis products of FBs. Moreover, there is only low levels (1-2 µg) of FBs that can be bound by this method (Krska *et al.*, 2007). IAC has been applied for the determination of several mycotoxins simultaneously using multiple antibodies (Wilcox *et al.*, 2015).

Toxicological studies with animals need relatively large quantities of pure mycotoxins. The loss during purification of the extract was reduced using the CPC purification method combined with ion exchange chromatography (Hübner *et al.*, 2012). The CPC method is a liquid-liquid chromatography technique that was developed to eliminate the problem of fumonisin loss during adsorption chromatography.

4. ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

ELISA is a biochemical technique based on the reaction between antigen and antibody as well as the reaction between enzyme and substrate. The result is based on the differences in spectroscopic behaviors of substrate and product molecules. Among the different sorts of techniques, i.e. direct, indirect, sandwich and competitive ELISA, the latter was applied most frequently to determine fumonisins because of its high sensitivity and specificity. Both indirect competitive ELISA (IC-ELISA) and direct competitive ELISA (DC-ELISA) were used to detect fumonisins. Competitive immunoassay is based on the distribution of enzyme-conjugated antibodies between protein bound hapten and free antigens in the sample extract. ELISA can be used for total fumonisin analysis and monoclonal antibodies can be also applied for the separation of fumonisin groups. Therefore, to determine certain fumonisins such as FB_1, FB_2, and FB_3, monoclonal antibodies (MAb) have to be produced (Azcona-Olivera *et al.*, 1992) and the standard curve of fumonisin concentration should be used for quantification (Vrabcheva *et al.*, 2002).

A brief procedure of DC-ELISA includes the following steps. First the microplate wells are coated by FB-MAb. After washing, the extracted sample and FB-HRP (horseradish peroxidase) are added simultaneously and coincubated. The second washing step is done before the addition of the substrate. The assay is stopped by a strong acid (H_2SO_4) and the absorbance is measured at 450 nm – 650 nm (Pestka *et al.*, 1994; Quan *et al.*, 2006).

The IC-ELISA approach is similar to DC-ELISA with some changes in the procedure. The ELISA plates are coated with FBs – ovoalbumin conjugate then blocked by a protein, e.g. casein

from skim milk. After washing by phosphate buffered saline (PBS), the extracted FBs sample or the FBs standard solution and FB-MAb were added. The second washing is applied and the addition of IgG conjugated with enzyme (IgG-HRP) is performed. The substrate solution is added and then the reaction is stopped subsequently by H_2SO_4. The optical density (OD) is determined by the reader using 450 nm wavelength (Ono *et al.*, 2000).

5. CHROMATOGRAPHIC METHODS

5.1. Thin layer chromatography (TLC)

TLC methods have been used for the detection of fumonisins since the 1990s. These methods are mainly applied to qualify the presence of mycotoxins. First, the samples are extracted and purified then the extract is evaporated (Rottinghaus *et al.*, 1992; Vrabcheva *et al.*, 2002; Mohanlall *et al.*, 2013) and dissolved in an acetonitrile:water mixture. The sample solutions and fumonisin standard solutions are spotted on a plate which is coated with a stationary phase. One side of the plate is immerged in a solvent, called eluent, which moves up the plate by capillary action. To develop the TLC method for determining fumonisins, various sorts of stationary phases and solvents have been applied (Table 2). The fumonisin levels can be determined by visual comparison with standards, using UV, fluorescence or other techniques.

Table 1. Functional groups of the fumonisin analogues
(adapted from Musser and Plattner, 1997)

Fumonisin	Carbon position				Formula
	C1	C2	C3	C10	
FA_1	CH_3	$NHCOCH_3$	OH	OH	$C_{36}H_{61}NO_{16}$
FA_2	CH_3	$NHCOCH_3$	OH	H	$C_{36}H_{61}NO_{15}$
FA_3	CH_3	$NHCOCH_3$	H	OH	$C_{36}H_{61}NO_{15}$
FB_1	CH_3	NH_2	OH	OH	$C_{34}H_{59}NO_{15}$
FB_2	CH_3	NH_2	OH	H	$C_{34}H_{59}NO_{14}$
FB_3	CH_3	NH_2	H	OH	$C_{34}H_{59}NO_{14}$
FC_1	H	NH_2	OH	OH	$C_{33}H_{57}NO_{15}$
FP_1	CH_3	3HP	OH	OH	$C_{39}H_{62}NO_{16}^+$
FP_2	CH_3	3HP	OH	H	$C_{39}H_{62}NO_{15}^+$
FP_3	CH_3	3HP	H	OH	$C_{39}H_{62}NO_{15}^+$

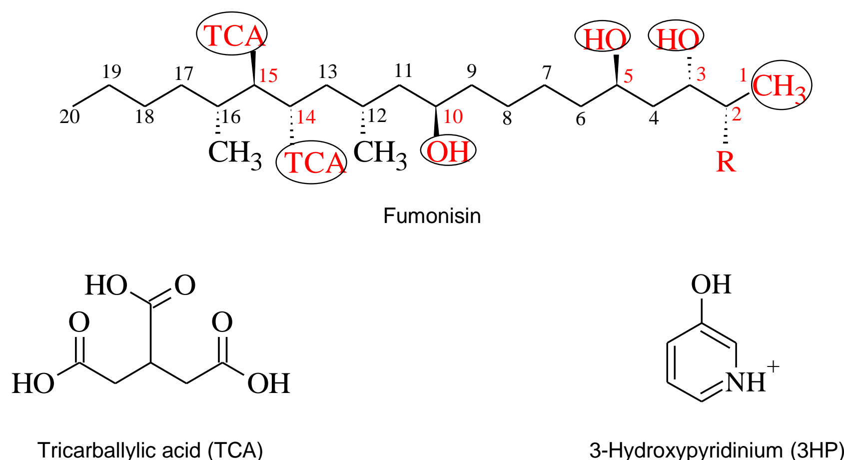

Figure 1. Chemical structure of fumonisins

Table 2. Conditions of Thin Layer Chromatographic (TLC) separation of fumonisins

Stationary phase		Solvent	Type of fumonisins	References
C18 reversed phase TLC plates	10 x 10 cm	Methanol:1% aqueous KCl (3:2, v/v)	FB_1, FB_2	Rottinghaus *et al.*, 1992
Silica gel 60 plates	20 x 10 cm	1-butanol:acetic acid:water (20:10:10, v/v/v)	FB_1	Dupuy *et al.*, 1993; Mohanlall *et al.*, 2013
C18 reversed phase TLC plates	No information	Ethanol:Water:Acetic acid (65:35:1)	FB_1	Schaafsma, 1998
C18 reversed phase TLC plates	20 x 20 cm	4% aqueous KCl:Methanol (3:7, v/v)	FB_1	Vrabcheva *et al.*, 2002
Aluminium sheet, silica gel TLC plate	No information	96% Methanol:Water (80:20, v/v)	FB_1, FB_2	Aboul-Nasr and Obied-Allah, 2013

Table 3. High Performance Liquid Chromatography (HPLC) conditions applied for the separation of fumonisins

Type of fumonisin	Samples	Instrument	Fluorescence (Excitation wavelength, emission wavelength)	Mobile phase	References
FB_1, FB_2	Grain-based foods	2150 LKB pump 7125 Rheodyne injector MPF-44 B fluorimetric detector	335 nm, 440 nm	Methanol:0.1 M NaH_2PO_4 (75:25, v/v), adjust to pH 3.35 by the addition of orthophosphoric acid.	Pestka *et al.*, 1994
FB_1, FB_2, FB_3	Corn	LC pump, C_{18} reverse phase column	335 nm, 440 nm	Methanol:0.1M NaH_2PO_4 (77:23, v/v), adjust to apparent pH 3.3 with H_3PO_4.	AOAC Official Method 995.15
FB_1, FB_2	Maize-based foods	Agilent Technologies SL 1200 Series, binary pump	343 nm, 445 nm	Methanol (A) and 0.1 M phosphate buffer (B) at pH 3.15 (B). The optimized elution gradient: 2 min 60% A and 40% B; 5 min 65% A and 35% B; 3 min to 75% A and 25% B; 2 min to the initial mobile phase composition, at which the system is re-equilibrated for 5 min. The flow rate is 0.8 ml min^{-1}.	Muscarella *et al.*, 2008
FB_1, FB_2, FB_3	Dry Figures	Agilent Technologies 1100 system	355 nm, 440 nm	Methanol:0.1M NaH_2PO_4. H_2O (77:23; v/v) solution, adjust to pH 3.35 with orthophosphoric acid.	Karbancioglu-Guler & Heperkan, 2009
FB_1, FB_2	Animal feeds, food samples, inoculated corn and rice	Waters Alliance HPLC system. Chromolith® performance RP-18e (100mm–4.6mm) column	335 nm, 440 nm	Methanol:0.1M dihydrogenphosphate (78:22, v/v), the mixture is adjusted to pH 3.35 with ortho-phosphoric acid.	Khayoon *et al.*, 2010
FB_1, FB_2	Corn masa flour	Agilent 1100 series binary pump	335 nm, 440 nm	Mixture of acetonitrile:acetic acid (99:1, v/v) (A) and water:acetic acid (99:1, v/v) (B). Program: 43% B for 5 mins then up to 54% at 21 min, 58% at 25 min and keep constant up to 30 min. The flow rate is 0.8 ml min^{-1}.	Girolamo *et al.*, 2011
FB_1, FB_2	Corns	Waters Binary model 1525 HPLC	355 nm, 440 nm	Methanol/0.1 M NaH_2PO_4 (75:25, v/v), adjust to pH 3.35 by the addition of phosphoric acid.	Aboul-Nasr & Obied-Allah, 2013

Table 4. Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS) conditions applied for the separation of fumonisins

Type of fumonisin	Samples	Instrument	Mobile phase of LC	MS/MS condition	References
FB_1, FB_2, FB_3	Corn-based foods	LC Alliance 2695 system; TQ mass spectrometer Quattro LC from Micromass	Water + 0.5% formic acid (A) and Methanol + 0.5% formic acid (B). An isocratic step of 65% B for 3 min, gradually increased to 95% B in 4 min and held constantly for 7 min. Flow rate is 0.5 ml min^{-1}	Positive ion mode. The (ESI) source values: capillary voltage, 3.20 kV; source temperature, 125°C; desolvation temperature: 300°C; desolvation gas: nitrogen, 99.99% purity, flow: 500 l/h.	D'Arco *et al.,* 2008; Silva *et al.*, 2009
FB_1	Bovine milk	LC Alliance 2695 system; Quattro Premier XE equipped with an ESCITM Multi-Mode Ionization Source	Water:acetonitrile (90:10, v/v) + 0.3% formic acid (A) and Acetonitrile + 0.3% formic acid (B). Isocratic conditions (75%A and 25%B)	Positive ion mode. The (ESI) source values: capillary voltage: 3.25 kV; source temperature: 140°C; desolvation temperature: 400°C.	Gazzotti *et al.*; 2009
FB_1, FB_2	Fresh corn	LC Alliance 2695 system. Waters Quattro MicroTM API triple-quadrupole MS	Methanol:water:formic acid (75:25:0.2, v/v/v)	Positive ion mode. The (ESI) source values: capillary voltage: 3.5 kV; source temperature: 120°C; desolvation temperature: 350°C. desolvation gas flow rate: 600 l/h.	Li *et al.*, 2012

5.2. High performance liquid chromatography (HPLC)

For HPLC analysis, the pressurized liquid solvent (the mobile phase) containing samples is pumped through a column filled with a solid adsorbent material (stationary phase). With the appropriate selection of the mobile phase and stationary phase, fumonisins elute from the column separately from each other and from the other components, and can be separately detected by a UV or fluorescence detector. However, because of the lack of fluorescence or UV absorbing chromophores, measurement of fumonisins is based on the derivatization of their free amino groups (Shephard, 1990). Several sorts of derivatization reagents have been applied for fumonisin Bs analysis such as *ortho*-phthaldialdehyde (OPA), naphthalene-2,3-dicarboxaldehyde (NDA) and dansyl chloride (DnS-Cl). OPA is the most commonly used reagent for fumonisin analysis in all kinds of matrices (Arranz *et al.*, 2004). DnS-Cl derivatization was reported as not appropriate for the determination of fumonisins from maize due to the low recovery (Ndube, 2011). Reverse phase HPLC (RP-HPLC) is applied more frequently for the determination of fumonisins (AOAC, 2000; Ono *et al.*, 2000; Bartók *et al.*, 2010a) than normal phase high performance liquid chromatography (NP-HPLC). The primary cause may be that fumonisins can be eluted and extracted by water that is usually part of the mobile phase in RP-HPLC. In order to optimize the composition of the mobile phase, using a silica-based monolithic column, Khayoon *et al.* (2010) investigated six different ratios of methanol: phosphate buffer and found the optimal ratio as 78:22 (v/v). The temperature and the flow rate of separation were also optimized (30 °C and 1.0 mL min^{-1}, respectively). A compilation of conditions applied to the HPLC analysis of fumonisins reported by other authors can be seen in table 3.

5.3. Gas chromatography – Mass spectrometry (GC-MS)

Analysis of fumonisin mycotoxins by GS-MS is based on the separation of their volatile derivatives such as trimethylsilyl (Jackson and Bennett, 1990) and triflioroacetate (Plattner *et al.*, 1990, 1994) derivatives. GC-MS analysis was also conducted by quantitation of trycarballylic acid formed during the alkaline hydrolysis of fumonisins (Syndenham *et al.*, 1990). The method was reported having high sensitivity, but one more procedure, hydrolysis, was necessary

prior to analysis. Owing to the nonvolatile characteristics of fumonisines and the necessity of one more step, derivatization or hydrolysis, GC-MS method is not used frequently for the quantitative analysis of fumonisins.

5.4. Liquid chromatography – Mass spectrometry (LC-MS)

LC-MS is one of the best methods for quantification of fumonisins because of its high sensitivity and accuracy. This technique combines the physical separation capabilities of LC with the mass analytic capabilities of MS. It had been extremely difficult to connect LC with MS before the 1990s because they require very different conditions such as temperature or volume of analytes. The atmospheric pressure ionization (API) effectively solved this problem. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) are the two main types of API interfaces. APCI is suitable for primarily low and medium polarity compounds whereas ESI is the most appropriate for ionic compounds with high polarity. Therefore, ESI was selected for fumonisin determination. To analyze fumonisin isomers, LC tandem mass spectrometry (LC-MS/MS) is usually used based on the better capability of separation and identification of compounds in complex mixtures. The analytical conditions applied in LC-MS/MS depend on the type of fumonisin and the type of samples (Table 4). The limit of quantification (LOQ) for FB_1 and FB_2 was 2 µg kg^{-1} (D'Arco *et al.*, 2008), while Silva *et al.* (2009) reported a higher LOQ value, 12 µg kg^{-1}, for fumonisins B1 and B2, using the same LC-MS/MS system and conditions for corn-based foods analysis. Their method was modified by using ultrasonic extraction, and LOQs for FB_1 and FB_2 were 11.7 µg kg^{-1} and 8.3 µg kg^{-1} respectively, from fresh corn samples (Li *et al.*, 2012). In order to identify fumonisins and qualify them in corn, Tamura *et al.* (2015) utilized LC-Orbitrap MS. LOQs for FA_1, FA_2 and FA_3 were 0.34 µg kg^{-1}, 1.98 µg kg^{-1} and 0.92 µg kg^{-1}, respectively. LC-Orbitrap MS analysis proved to be better than LC-MS/MS regarding the detection of fumonisins at very low levels, as LOQs were between 0.05 and 0.12 µg kg^{-1} for FBs.

6. COMPARISON BETWEEN FUMONISIN ANALYTIC METHODS

The results of fumonisin analyses conducted with DC-ELISA, GC-MS and HPLC were compared in grain-based foods. Correlations between the measured concentrations of fumonisin comparing ELISA and GC-MS, ELISA and HPLC and GC-MS and HPLC were 0.478, 0.512 and 0.946, respectively (Pestka *et al.*, 1994). The same rice sample was analyzed simultaneously with DC-ELISA and HPLC. The concentrations measured with HPLC were slightly higher than that of DC-ELISA. The total fumonisin levels of positive samples ranged from 2.3 to 5.8 µg/g by HPLC while the fumonisin levels ranged from 1.9 to 3.6 µg/g by ELISA (Abbas *et al.*, 1998). On the contrary, the detected FB_1 levels in dry Figures by ELISA was much higher than by HPLC, a range of 0.16 - 108.34 µg g^{-1} compared with 0.046 - 0.100 µg g^{-1}, respectively (Karbancioglu - Guler & Heperkan, 2009).

The advantages and disadvantages of methods were discussed in a previous review (Pascale, 2009). However, the Pascale's article mentioned mycotoxins in general. As for fumonisin analysis, the utilization levels of several methods are discussed in more detail in Sections 4 and 5. ELISA is rapid, sensitive and easy to apply both in laboratory and field environments. The TLC method also requires less instrumentation if the plates are analyzed visually (Vrabcheva *et al.*, 2002). These methods are usually applied to screen and qualify mycotoxins. GS-MS is hardly ever applied because of the nonvolatile characteristics of fumonisins. In order to determine the exact concentration of analytes, HPLC-MS and LC-MS/MS are mainly used.

7. THE TRENDS OF THE DETECTION OF FUMONISINS IN THE FUTURE

Though several methods have been applied for the analysis of fumonisins, the techniques still are developing to increase sensitivity and

accuracy. The recent trend of determination of fumonisins has utilized sensors which are crucial to detect mycotoxin molecules (Chauhan *et al.*, 2016). The sensors, i.e. aptasensors or immunosensors, allow detecting fumonisin at very low concentrations. By using an aptasensor, the range of FB_1 detection was from 0.01 to 100 ng ml^{-1} with a detection limit of 0.01 ng ml^{-1} (Wu *et al.*, 2013). The fumonisin B_1 detection level was from 0.01 to 1000 ng ml^{-1} with a detection limit of 2 pg ml^{-1} under optimised conditions using the immunosensor application (Yang *et al.*, 2015).

Furthermore, all methods are being developed in parallel to detect fumonisin with other types of mycotoxins. The inevitable trend will be more popular shortly for several reasons: foods and feeding stuffs are contaminated by many types of mycotoxins, the chemical structure of mycotoxins are various in several derivatives, and the combined effect of fumonisin and other kinds of mycotoxins on cells is more complex than the effect of only fumonisin. From this point of view, research groups are developing methods to detect multi-mycotoxins (Tamura *et al.*, 2011; Jia *et al.*, 2014; Liao *et al.*, 2015).

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