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Free and hidden fumonisins in Brazilian raw maize samples

Maurício S. Oliveira [a], Andressa C.L. Diel [a], Ricardo H. Rauber [b], Fabiana P. Fontoura [a], Adriano Mallmann [a], Paulo Dilkin [a], Carlos A. Mallmann [a, *]

[a] Universidade Federal de Santa Maria (UFSM), Laboratório de Análises Micotoxicológicas (LAMIC), CEP 97105 900, Santa Maria, Rio Grande do Sul, Brazil
[b] Programa de Pós-graduação em Zootecnia, Universidade Tecnológica Federal do Paraná (UTFPR), Bolsista de Pós-doutorado em empresa Fundação Araucária e Coordenação de Aperfeiçoamento de Pessoal de Nível de Superior (CAPES), CEP 80610 190, Curitiba, Paraná, Brazil

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ABSTRACT

Fumonisins are secondary metabolites produced primarily by fungi strains that belong to the genera *Fusarium* and *Alternaria*, which have been shown to be highly prevalent in maize crops. Some authors have documented the presence of hidden forms of fumonisins occurring in raw maize. This purpose of this study was to determine the occurrence of free and hidden fumonisins in raw maize. The concentrations of fumonisins in 72 naturally contaminated maize samples were analyzed using liquid chromatography coupled to mass spectrometry. The performance parameters of the method to determine free fumonisins forms (FB_1 and FB_2) and hydrolyzed fumonisins forms (HFB_1 and HFB_2) were evaluated using the standards from the Commission of the European Communities (Commission, 2002). The analytical methods employed fell within the established guidelines. The amount of total fumonisins measured based on the hydrolyzed forms ($HFB_1 + HFB_2$) was 1.5–3.8 times greater than the amount of free fumonisins ($FB_1 + FB_2$). The concentration of hidden fumonisins was calculated by subtracting the levels of free fumonisins from the total fumonisin levels. The levels of hidden fumonisins were calculated to be 0.5–2.0 times greater than the level of free fumonisins. A strong positive correlation ($R = 0.97$) was observed between free fumonisins ($FB_1 + FB_2$) and. total fumonisins ($HFB_1 + HFB_2$). Based on this correlation, a predictive model was generated to estimate the total fumonisin level based on the measured/reported free fumonisin concentration. These results show that the risk of exposure to fumonisins is likely underestimated if only free fumonisins are considered. However, the predictive model could be a novel approach to estimating the total amount of fumonisins in maize samples without needing to perform expensive and time-consuming analytical methods.

1. Introduction

Fumonisins are secondary metabolites formed in maize before harvest by fungi strains in the genera *Fusarium* and *Alternaria* (Bezuidenhout et al., 1988). *Fusarium verticillioides* and *F. proliferatum* can produce fumonisins analogs grouped into serie B and C and are the main sources of fumonisins in maize (Berthiller, Schuhmacher, Adam, & Krska, 2009, Berthiller et al.,2013; Bezuidenhout et al., 1988). The fumonisins analogs can be classified into four main groups, A, B, C and P, all sterified by tricarballylic acid (TCA). Fumonisins analogs grouped into serie X, are sterified by other carboxillic acids such as cis-aconitic acid, oxalylsuccinic acid and oxalylfumaric acid (Bartók, Szécsi, Szekeres, Mesterházy, & Bartók, 2006). Fumonisin B_1 (FB_1) and Fumonisin B_2 (FB_2) are the most abundant fumonisins in maize and therefore, they have been the most studied (Shephard, Thiel, Stockenstrom, & Sydenham, 1996).

The chemical structure of fumonisins is characterized by a polyhydroxy alkyl amine chain, diesterified with molecules of tricarballylic acid (Bezuidenhout et al., 1988; Dall'Asta, Galaverna, et al., 2009). Fumonisins are structural analogs of sphingoid bases and can inhibit the ceramide synthetase enzyme involved in the biosynthesis of sphingolipids. This disrupts the normal metabolism of these bases, which is recognized as the mechanism for fumonisin induced damage in animals and humans (Voss, Smith, & Haschek, 2007; Wang, Norred, Bacon, Riley, & Merrill, 1991).

FB_1 causes different toxic responses in human and animals. Studies have associated an increased risk of human esophageal cancer with the consumption of maize contaminated with FB_1 (Kim, Scott, & Lau, 2003; Rheeder et al., 1992) and DNA damage in

Abbreviations: FB_1, fumonisin B_1; FB_2, fumonisin B_2; HFB_1, Hydrolyzed fumonisin B_1; HFB_2, Hydrolyzed fumonisin B_2.

* Corresponding author. Tel.: +55 55 3220 8445.
E-mail address: mallmann@lamic.ufsm.br (C.A. Mallmann).

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human fibroblasts (Kim et al., 2003). Given that it has carcinogenic effects, the International Agency for Research on Cancer (IARC) has classified FB_1 in group 2B, as a possible carcinogenic compound for humans. FB_1 has different effects in animals, like leucoencephalomalacea in horses, pulmonary edema in swine (Haschek, Gumprecht, Smith, Tumbleson, & Constable, 2001; Voss et al., 2007), and carcinogenic effects in several animal species (Muller, Dekant, & Mally, 2012). It has also been reported to be detrimental to broiler's performance and physiological responses (Broomhead, Ledoux, Bermudez, & Rottinghaus, 2002; Ledoux, Brown, Weibking, & Rottinghaus, 1992; Rauber et al., 2013).

Hidden fumonisins were long believed to occur only after food processing (Berthiller et al., 2009; Falavina, Cirlini, Galaverna, & Dall'Asta, 2012). However, Dall'Asta, Falavigna, Galaverna, Dossena, and Marchelli (2010) showed that hidden fumonisins can also be found in unprocessed maize. The exact chemical reactions, which are responsible for the occurrence of naturally hidden forms, are still unknown (Berthiller et al., 2009, 2013; Dall'Erta et al., 2013). Hidden fumonisins cannot be directly analyzed and have to be released from the matrix by a sample treatment that converts them into extractable forms (Berthiller, Sulyok, Krska, & Schuhmacher, 2007; Galaverna, Dall'Asta, Mangia, Dossena, & Marchelli, 2009; Seefelder, Knecht, & Humpf, 2003).

Understanding that there are hidden fumonisins in raw maize suggests that the total content of fumonisins might be underestimated if the sample is inappropriately prepared before undertaking a chromatographic analysis. Several authors have reported the use of an alkaline hydrolysis step to prepare food samples to determine the total content of fumonisins (Dall'Asta, Galaverna, Aureli, Dossena, & Marchelli, 2008; Kim et al., 2003; Park, Scott, Lau, & Lewis, 2004). The unesterified polyols obtained from FB_1 and FB_2 after the alkaline hydrolysis are called hydrolyzed FB_1 (HFB_1) and hydrolyzed FB_2 (HFB_2), respectively (Galaverna et al., 2009; Park et al., 2004). Hidden fumonisins are indirectly measured as the difference between total fumonisins estimated by the hydrolyzed forms and free forms (Park et al., 2004). In 2011, the Brazilian Health Surveillance Agency (ANVISA), proposed a maximum tolerated level (MTL) for $FB_1 + FB_2$ of 2500 μg/kg for maize flour, maize cream, fubá, flakes, canjica, and canjiquinha, and 2000 μg/kg for maize starch and maize based products (Brasil, 2011). Starting in 2014, ANVISA proposed an MTL for raw maize intended for further processing of 5000 μg/kg. All of these regulations are recent and none of them address hidden fumonisins. The aim of this study was to determine the total concentration of fumonisins based on the levels of HFB_1 and HFB_2, free FB_1 and FB_2, and hidden fumonisins in raw maize samples. Moreover, it was propose the use of a correlation equation as a predictive model to estimate the concentration of total fumonisins in samples that have already been analyzed.

2. Material and methods

2.1. Chemicals and Certified Reference Material

Purified FB_1 and FB_2 standards were purchased from Sigma–Aldrich (São Paulo, Brazil). Potassium hydroxide, formic acid, and all solvents used (LC Grade), were purchased from J.T.Baker (São Paulo, Brazil). Ultra-pure water was obtained from a Milli-Q System (Millipore, USA). Maize flour contaminated with FB_1 (591 μg/kg) and FB_2 (305 μg/kg) was provided by the Food Analysis Performance Assessment Scheme (FAPAS) (Certified Reference Material (CRM) number 2287) and used to evaluate the accuracy of the FB_1 and FB_2 measured and the sum of FB_1 and FB_2. HFB_1 and HFB_2 standards were prepared by alkaline hydrolysis following the technique described by Dall'Asta et al. (2008).

2.2. Samples

Seventy-two naturally contaminated maize samples were collected from different locations in the state of Rio Grande do Sul, Brazil, between 2012 and 2013. Samples were automatically collected following the sampling protocol published by Mallmann et al. (2013). At the laboratory, samples were ground in a ZM 200 ultra-centrifugal mill (Retsch, Germany) and partitioned with a PT 100 sample divider (Retsch, Germany). The analysis of free FB_1 and FB_2, and HFB_1 and HFB_2 were performed in duplicate.

2.3. Analysis of free fumonisins (FB_1 and FB_2)

Ten grams of maize sample was ground through a 2.0 mm screen and extracted with 50 mL water/acetonitrile (1:1, v/v) for 5 min in a high-speed blender. The extract was then filtered. An aliquot of 20 μL was diluted in a 1% formic acid acetonitrile/water solution (1:1, v/v) before liquid chromatography-mass spectrometry (LC-MS/MS) analysis.

2.4. Analysis of hydrolyzed forms (HFB_1 and HFB_2) and total fumonisins ($HFB_1 + HFB_2$)

Ten grams of maize sample were ground through a 2.0 mm screen and submitted to alkaline hydrolysis with 100 mL of KOH 2 M at room temperature for 10 min in a high-speed blender. After blending, 100 mL of acetonitrile was added and stirred for 30 min in a shaker at 80 rpm. An aliquot of 15 mL was dried under nitrogen flow and resuspended in 1.5 mL of 1% formic acid acetonitrile/water solution (1:1, v/v). After resuspension, the sample was filtered with a 0.45 μm filter and analyzed by LC-MS/MS. The concentration of total fumonisins was determined based on the analysis of the hydrolyzed forms after alkaline hydrolysis preparation (Dall'Asta et al., 2008). The content of hidden fumonisins was estimated based on the difference between total and free fumonisins (Kim et al. 2003; Park et al. 2004).

2.5. Liquid chromatography-mass spectrometry method (LC-MS/MS)

Free and hydrolyzed fumonisins were determined using the HPLC Agilent 1200 (Agilent Technologies Inc., USA) equipped with an API5000 triple quadruple mass spectrometer with an electrospray source (AB Sciex, Canada). The LC column was a Zorbax® C_{18}, 150 × 4.6 mm, 5 μm (Agilent Technologies Inc., USA) column with a C_{18} pre-column cartridge, run under a flow rate of 0.8 mL/min, at a column temperature of 40 °C, and an injection volume of 5 μL. A gradient elution was performed using water (eluent A) and acetonitrile (eluent B) both acidified with 1% formic acid: 0–3.5 min isocratic step 35% B; 3.5–6 min to 70% B; 6–8 min isocratic step 70% B; a re-equilibration step at 35% B for 2 min to return to the initial condition. The MS source dependent parameters were: curtain gas (CUR) 20 L/min; collision-activated dissociation gas (CAD) was set to medium; source temperature 650 °C, dry gas 1 (GS1) 50 L/min, dry gas 2 (GS2) 45 L/min, and the spray voltage was set to 5500 V. Detection was performed in positive mode (ESI+) using a multiple reaction monitoring (MRM) mode, by monitoring two transitions for each analyte, the primary transition was used for quantification and the second transition was used for confirmation (Table 1).

2.6. Method performance parameters

Performance parameters were evaluated using the standards from the Commission of the European Communities (Commission, 2002). For the free forms, the amounts of spiked fumonisins in a sample were determined as described in Section 2.3. For the bound

Table 1
Retention time and compound dependent parameters for LC-ESI-MS/MS analysis of free and hydrolyzed fumonisins.

Compound	Retention time (min)	Precursor ion (m/z)$[M+H]^+$	Declustering potential (V)	Product ions (m/z)[a]	Collision energy (V)[a]	Cell exit potential (V)[a]
FB_1	3.5	722.5	160	334.4/704.4	57/43	18/20
FB_2	7.1	706.5	160	336.4/688.4	51/41	18/20
HFB_1	2.7	406.5	140	334.5/370.5	33/30	21/21
HFB_2	6.8	390.5	140	318.5/354.5	32/26	25/28

[a] Numerical values are given in the order quantifier/qualifier ion.

forms, the spiked samples underwent the alkaline hydrolysis procedure and then the hydrolyzed forms were determined. For all of the compounds linearity and calibration curves were evaluated based on the calibration curve at six concentration levels ranging from 125 to 10,000 μg/kg. The method accuracy, defined as percent recovery (%), and precision, defined as percent of coefficient of variation, were evaluated using experiments to measure the recovery of fumonisins spiked into blank maize samples at three different concentrations (125; 2000 and 10,000 μg/kg) that covered the linear range, seven samples per level, on three different days by two different analysts. Detection limits (LODs) and quantification limits (LOQs) were estimated by spiking the blank maize samples with each analyte (FB_1, FB_2, HFB_1, and HFB_2). The LOD and LOQ were calculated at a signal to noise ratio of 3:1 and 10:1 respectively. A simple correlation was used to assess the relationship between the levels of free and total fumonisins obtained from the maize samples and a predictive equation was proposed. The predictive equation was applied to results obtained from raw maize samples analyzed for free FB_1 and FB_2 over the last four years (2010–2013) at the Laboratório de Análises Micotoxicológicas located at Universidade Federal de Santa Maria (LAMIC/UFSM). The number of samples analyzed by year was 3,790, 4,965, 3,643, and 2285 respectively.

2.7. Statistical analysis

The results of the total free fumonisins and total fumonisins after hydrolysis were compared by Tukey's test ($P \leq 0.05$) within samples. The data were also submitted to simple correlation analysis to verify the association between free and total fumonisins. A predictive equation was proposed to estimate the total amount of fumonisins based on the amount of free ($FB_1 + FB_2$) fumonisins. Statistical analysis was performed using the Statgraphics Centurion computer statistical program (Statgraphics Centurion 15.2.14, Manugistics Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Method performance parameters

The accuracy of the technique used to measure fumonisins was evaluated using recovery experiments (purified analytes in blank maize) and the CRM sample. For the recovery experiments the intra-day precision for FB_1, FB_2, HFB_1, and HFB_2 were 98.8, 99.2, 91.2, and 93.2, respectively. According to the commission decision 2002/657/EC for experiments adding purified standards to samples at levels >10 μg/kg, the recovery range established must be between 80% and 110%. Thus, our measured recovery rates fall into the appropriate range. The results for the CRM of maize were reported as *z-scores*. For the sample results to be considered in agreement with the established reference compound, the scores had to be between −2.0 and 2.0. For the reference maize sample, the *z-scores* for FB_1 (0.4), FB_2 (0.3), and $FB_1 + FB_2$ (0.4) were within this range. The precision of the method was evaluated using the percent coefficient of variation (CV(%)). The inter-day precision results for FB_1, FB_2, HFB_1, and HFB_2 were 1.6, 2.5, 6.4, and 7.2, respectively. The commission (2002) states that the CV(%) must be below the level calculated by the Horwitz equation. For the fumonisin levels used in this study, the CV(%) recommended is 21.8 (125 μg/kg), 14.4 (2000 μg/kg), and 11.3 (10,000 μg/kg). All of the results from the recovery experiment were in accordance with this standard. The detection limits and quantification limits were 10/125, 20/125, 35/125, and 40/125 μg/kg for FB_1, FB_2, HFB_1, and HFB_2 respectively.

3.2. Analysis of free and hidden fumonisins in maize samples

In all of the samples analyzed the concentration of total fumonisins was higher than free fumonisins. The concentration of total fumonisins was calculated based on the sum of HFB_1 and HFB_2 The levels of total fumonisins ($HFB_1 + HFB_2$) and total free fumonisins ($FB_1 + FB_2$) measured in each maize sample were compared using the Tukey's test and in all cases, they were significantly different ($P \leq 0.05$). The increase in the concentration of fumonisins is due to the release of hidden forms during the hydrolysis procedure. Six samples (8%) were thought to be free of fumonisins; however following hydrolysis, the concentration of total fumonisins was greater than zero. This is interesting because FB_1 has low bioavailability and absorption at gastrointestinal level, and this may explain the occurrence of toxic effects even when feed contaminated with low doses is consumed (Marasas, Miller, Riley, & Visconti, 2000; Shier, 2000). The presence of hidden fumonisins, even in maize samples that lack free fumonisins, could support the hypothesis that fumonisins associated with carbohydrates and proteins (like the hidden fumonisins) can be preferentially absorbed, and then return to the free form and cause toxic effects (Dall'Asta et al., 2008).

The ranges of concentrations of total fumonisins ($HFB_1 + HFB_2$) found in this study were 1.5–3.8 times the concentration of free $FB_1 + FB_2$. Hidden fumonisins were also present in all analyzed samples at a range of concentrations between 0.5 and 2.0 times the concentration of free fumonisins. When the measured amount of total free fumonisins ($FB_1 + FB_2$) was compared with the MTL allowed by ANVISA (5000 μg/kg), 19 samples (26%) exceeded the suggested regulations. However, if the total concentration of fumonisins, including the hidden fumonisins was considered, the number of samples that exceeded the suggested regulations increased to 56 samples (78%). This represented a 52% increase in the number of samples that were not recommended for consumption. The established MLT is based on concerns regarding the toxicological effects caused by mycotoxins in humans and animals. A new MLT should be considered if the presence of hidden forms of fumonisins can be estimated for regulatory purposes (Berthiller et al., 2009; Wagacha & Muthomi, 2008).

Based on the results of free fumonisins ($FB_1 + FB_2$) and total fumonisins ($HFB_1 + HFB_2$) obtained from the 72 samples in this study, a correlation equation was established to predict the total concentration of fumonisins in samples based on the results on the free fumonisins analysis (Fig. 1). Analysis of the free forms of FB_1 and FB_2 is routinely performed in many laboratories, it is higher throughput, cheaper, and less time consuming than analyzing the hydrolyzed forms (Dall'Asta, Mangia, et al., 2009; Kim et al., 2003;

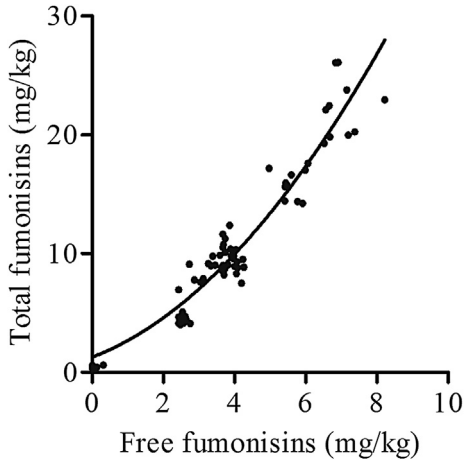

Fig. 1. Correlation between the concentration of free (FB_1 + FB_2) and total (HFB_1 + HFB_2) fumonisins based on the determination of hydrolyzed forms in raw maize samples. The equation for this correlation was: *Total fumonisins = (0.8583 + 0.5615*Free fumonisins)^2*; R = 0.97.

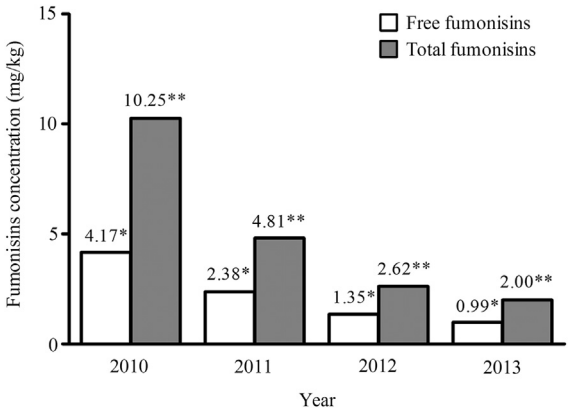

Fig. 2. The relationship between contamination by free fumonisins and the estimated total fumonisins in maize samples from the LAMIC/UFSM database. * Average contamination of free fumonisins (FB_1 + FB_2) ** Estimated total fumonisin concentration. The concentration of total fumonisin for each year was estimated using the correlation equation in Fig. 1.

Sulyok, Krska, & Schuhmacher, 2007). Given that six samples that had no free fumonisins (FB_1 + FB_2) had measurable levels of HFB_1 and HFB_2 after the hydrolysis step, the equation was developed using the conditions that free fumonisins (FB_1 + FB_2) = 0 and total fumonisins (HFB_1 + HFB_2) ≥ 0. Consistent with the findings from Dall'Asta et al. (2010), who proposed a linear correlation model to predict the total amount of fumonisins, our study found a strong positive correlation between the amount of free fumonisins forms and the amount of total fumonisins our study found a strong positive correlation between the amount of free fumonisins forms and the amount of total fumonisins (R = 0.97 and P = 0.00). The final correlation equation was: *Total fumonisins = (0.8583 + 0.5615*Free fumonisins)^2*.

3.3. Retrospectively estimating the total fumonisin concentration in maize samples from 2010 to 2013

The correlation equation was applied to data regarding fumonisin contamination in maize stocks retrieved from the LAMIC/UFSM database. The average contamination from free fumonisins over the previous four years (2010–2013) was used to estimate the average concentration of total fumonisins contamination by year (Fig. 2). In 2010, the amount of total fumonisins was estimated to be 2.4 times the amount of free fumonisins. The total level of fumonisin contamination estimated decreased in 2011 (2.02-times) and 2012 (1.94-times) compared to the concentration of free fumonisins. However, in 2013, an increase for total fumonisins from 2012 was observed from 1.94-times to 2.02-times the amount of free fumonisins. These results indicated that the occurrence of hidden fumonisins was directly proportional to the occurrence of free fumonisin.

4. Conclusion

The performance parameters of the analytical methods used to quantify FB_1, FB_2, HFB_1, and HFB_2 met the standards in commission, (2002). The amount of hidden fumonisins occurring in raw maize samples was strongly correlated with the amount of free fumonisins. Thus, if only measure the level of free fumonisins, it can expect to underestimate the overall risk of fumonisin exposure. While most of the samples analyzed did not exceed the limits for fumonisins in Brazil, this did not account for the hidden forms of fumonisins that were not considered in regulatory limits.

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References

Bartók, T., Szécsi, A., Szekeres, A., Mesterházy, A., & Bartók, M. (2006). Detection of new fumonisin mycotoxins and fumonisin-like compounds by reversed-phase high-performance liquid chromatography/electrospray ionization ion trap mass spectrometry. *Rapid Communications in Mass Spectrometry, 20*(16), 2447–2462.

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S. D., Haesaert, G., Karlovsky, P., et al. (2013). Masked mycotoxins: a review. *Molecular Nutrition & Food Research, 57*(1), 165–186.

Berthiller, F., Schuhmacher, R., Adam, G., & Krska, R. (2009). Formation, determination and significance of masked and other conjugated mycotoxins. *Analytical and Bioanalytical Chemistry, 395*(5), 1243–1252.

Berthiller, F., Sulyok, M., Krska, R., & Schuhmacher, R. (2007). Chromatographic methods for the simultaneous determination of mycotoxins and their conjugates in cereals. *International Journal of Food Microbiology, 119*(1–2), 33–37.

Bezuidenhout, S. C., Gelderblom, W. C. A., Gorstallman, C. P., Horak, R. M., Marasas, W. F. O., Spiteller, G., et al. (1988). Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. *Journal of the Chemical Society-Chemical Communications*, 743–745.

Brasil. (2011). *Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC No 7, de 18 de fevereiro de 2011. Dispõe sobre limites máximos tolerados (LMT) para micotoxinas em alimentos*. Brasília, DF, Brasil: Diário Oficial da União – Ministério da Saúde. de 22/03/2011. Retrieved from http://portal.anvisa.gov.br/wps/wcm/connect/bc17db804f45fe2cbd41fdd785749fbd/Resolu%C3%A7%C3%A3o+0-2011-CGALI.pdf?MOD=AJPERES.

Broomhead, J. N., Ledoux, D. R., Bermudez, A. J., & Rottinghaus, G. A. (2002). Chronic effects of fumonisin B_1 in broilers and turkeys fed dietary treatments to market age. *Poultry Science, 81*, 56–61.

Commission of the European Communities. (2002). 2002 Commission of the European communities commission regulation (EC) No. 657/2002 of 12 August 2002 concerning the performance of analytical methods and the interpretation of results. *Official Journal of European Union, L, 221*, 8–29.

Dall'Asta, C., Falavigna, C., Galaverna, G., Dossena, A., & Marchelli, R. (2010). In vitro digestion assay for determination of hidden fumonisins in maize. *Journal of Agricultural and Food Chemistry, 58*(22), 12042–12047.

Dall'Asta, C., Galaverna, G., Aureli, G., Dossena, A., & Marchelli, R. (2008). A LC/MS/MS method for the simultaneous quantification of free and masked fumonisins in maize and maize-based products. *World Mycotoxin Journal, 1*(3), 237–246.

Dall'Asta, C., Galaverna, G., Mangia, M., Sforza, S., Dossena, A., & Marchelli, R. (2009). Free and bound fumonisins in gluten-free food products. *Molecular Nutrition & Food Research, 53*(4), 492–499.

Dall'Asta, C., Mangia, M., Berthiller, F., Molinelli, A., Sulyok, M., Schuhmacher, R., et al. (2009). Difficulties in fumonisin determination: the issue of hidden fumonisins. *Analytical and Bioanalytical Chemistry, 395*(5), 1335–1345.

Dall'Erta, A., Cirlini, M., Dall'Asta, M., Del Rio, D., Galaverna, G., & Dall'Asta, C. (2013). Masked mycotoxins are efficiently hydrolyzed by human colonic microbiota releasing their aglycones. *Chemical Research in Toxicology, 26*(3), 305–312.

Falavina, C., Cirlini, M., Galaverna, G., & Dall'Asta, C. (2012). Masked fumonisins in processed food: co-occurrence of hidden and bound forms and their stability under digestive conditions. *World Mycotoxin Journal, 5*(3), 325–334.

Galaverna, G., Dall'Asta, C., Mangia, M. A., Dossena, A., & Marchelli, R. (2009). Masked mycotoxins: an emerging issue for food safety. *Czech Journal of Food Sciences, 27*, S89–S92.

Haschek, W. M., Gumprecht, L. A., Smith, G., Tumbleson, M. E., & Constable, P. D. (2001). Fumonisin toxicosis in swine: an overview of porcine pulmonary edema and current perspectives. *Environmental Health Perspectives, 109*(2), 251–257.

Kim, E. K., Scott, P. M., & Lau, B. P. (2003). Hidden fumonisin in corn flakes. *Food Additives Contaminants, 20*(2), 161–169.

Ledoux, D. R., Brown, T. P., Weibking, T. S., & Rottinghaus, G. E. (1992). Fumonisin toxicity in broiler chicks. *Journal of Veterinary Diagnostic Investigation, 4*, 330–333.

Mallmann, A. O., Marchioro, A., Oliveira, M. S., Minetto, L., Wovst, L. R. S., Rauber, R. H., et al. (2013). Dois planos de amostragem para análise de fumonisinas em milho. *Ciência Rural, 43*(3), 551–558.

Marasas, W. F. O., Miller, J. D., Riley, R. T., & Visconti, A. (2000). *Environmental health criteria 219: Fumonisin B_1*. Geneva: World Health Organization.

Muller, S., Dekant, W., & Mally, A. (2012). Fumonisin B_1 and the kidney: modes of action for renal tumor formation by fumonisin B_1 in rodents. *Food and Chemical Toxicology, 50*(10), 3833–3846.

Park, J. W., Scott, P. M., Lau, B. P., & Lewis, D. A. (2004). Analysis of heat-processed corn foods for fumonisins and bound fumonisins. *Food Additives & Contaminants, 21*(12), 1168–1178.

Rauber, R. H., Oliveira, M. S., Mallmann, A. O., Dilkin, P., Mallmann, C. A., Giacomini, L. Z., et al. (2013). Effects of fumonisin B_1 on selected biological responses and performance of broiler chickens. *Pesquisa Veterinária Brasileira, 33*(9), 1081–1086.

Rheeder, J. P., Marasas, W. F. O., Thiel, P. G., Sydenham, E. W., Shephard, G. S., & Van Schalkwyk, D. J. (1992). *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology, 82*(3), 353–357.

Seefelder, W., Knecht, A., & Humpf, H. U. (2003). Bound fumonisin B_1: analysis of fumonisin-B_1 glyco and amino acid conjugates by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of Agricultural Food Chemistry, 51*(18), 5567–5573.

Shephard, G. S., Thiel, P. G., Stockenstrom, S., & Sydenham, E. W. (1996). Worldwide survey of fumonisin contamination of corn and corn-based products. *Journal of AOAC International, 79*(3), 671–687.

Shier, W. T. (2000). The fumonisin paradox: a review of research on oral bioavailability of fumonisin B(1), a mycotoxin produced by *Fusarium moniliforme*. *Journal of Toxicology-Toxin Reviews, 19*(2), 161–187.

Sulyok, M., Krska, R., & Schuhmacher, R. (2007). A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. *Analytical and Bioanalytical Chemistry, 389*(5), 1505–1523.

Voss, K. A., Smith, G. W., & Haschek, W. M. (2007). Fumonisins: toxicokinectics, mechanism of action and toxicity. *Animal Feed Science and Technology, 137*(3–4), 299–325.

Wagacha, J. M., & Muthomi, J. W. (2008). Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology, 124*(1), 1–12.

Wang, E., Norred, W. P., Bacon, C. W., Riley, R. T., & Merrill, A. H. (1991). Inhibition of sphingolipid biosynthesis by fumonisins. Implications for diseases associated with Fusarium moniliforme. *Journal of Biological Chemistry, 266*, 14486–14490.