772 J. Agric. Food Chem. **2011,** 59, 772–776
DOI:10.1021/jf103855x

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY
ARTICLE

Occurrence of Fumonisins B_2 and B_4 in Retail Raisins

PETER BOLDSEN KNUDSEN, JESPER MØLGAARD MOGENSEN,
THOMAS OSTENFELD LARSEN, AND KRISTIAN FOG NIELSEN*

Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark,
Søltofts Plads, Building 221, DK-2800 Kgs. Lyngby, Denmark

Concerns that raisins may be contaminated by fumonisins stem from the persistent occurrence of *Aspergillus niger* spores on raisins and the recent discovery of fumonisin production by *A. niger* on grapes, which leads to the widespread occurrence of fumonisin B_2 in wine. This study presents an LC-MS/MS survey of fumonisins in retail raisins. In 10 of 21 brands collected in Denmark, Germany, and The Netherlands, fumonisins B_2 and B_4 were detected at levels up to 13 and 1.3 μg/kg, respectively. Only fumonisin B_2 has been detected in wine, so the presence of fumonisin B_4 in raisins suggests that the fumonisins are produced mainly during the drying process concomitant with the decreasing water activity. Analysis of multiple packages from one manufacturer showed a 3-fold package-to-package variation, suggesting that a few raisins per package are contaminated.

KEYWORDS: Fumonisins B_2 and B_4; wine; grapes; cation exchange; SPE; solid-phase extraction

INTRODUCTION

Black aspergilli are distributed globally and occur on a great variety of substrates. They are also widely used in the food and biotechnology industries for the production of organic acids, enzymes, and other products (*1*).

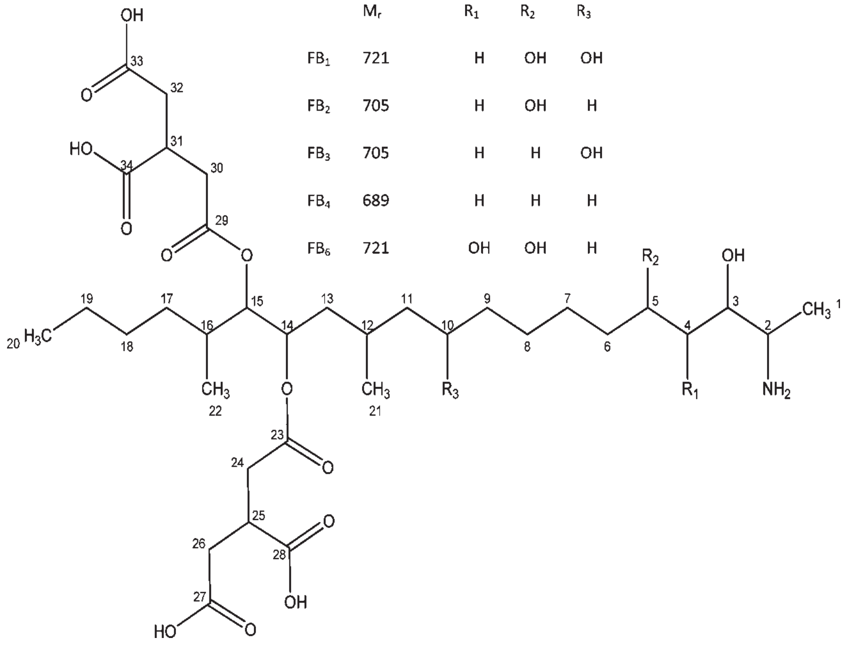
Black aspergilli are commonly found on the surface of healthy grapes during all growth stages (*2*). *Aspergillus* species are present on grape clusters early in the season, and their frequency increases in the later growth stages of the grapes (*3*). Of the various *Aspergillus* species, *Aspergillus niger* is by far the most commonly found on grapes and is shown in one study to occur on > 80% of samples (*4*). Although *A. niger* is the predominant species, *Aspergillus carbonarius* is the most problematic because it consistently produces high amounts of ochratoxin A, whereas only 8−40% of *A. niger* strains produce low amounts of this toxin (*5*,*6*). This is the main mycotoxin-related health concern in grape-derived products. Nonetheless, *A. niger* production of fumonisins B_2, B_4, and B_6 (*7*−*9*) (**Figure 1**) and the high frequency with which fumonisin B_2 occurs in wine (*10*, *11*) raise concerns about the possible widespread presence of fumonisins in raisins. These concerns are further fuelled by the production of fumonisins B_2 and B_4 on artificially infected grapes and raisins to levels as high as 8 mg/kg (*12*,*13*), as well as a study that found *A. niger* spores on all raisins investigated (*13*). A recent analysis of seven raisin samples from around the world claimed fumonisin levels as high as 35 mg/kg, mainly for fumonisin B_2, but also detected fumonisins B_1, B_3, and B_6, as well as a number of tentatively identified fumonisin B_1 and B_2 OH-positional isomers (*14*). The finding of fumonisins B_1 and B_3 is unusual, because the *FUM2* gene encoding the enzyme for a C-10 hydroxylation that is required for the production of these isomers has not been detected in any of the three available full-genome sequences for *A. niger* strains (*15*, *16*). Furthermore, no other studies on fumonisin production in *A. niger* have detected fumonisin B_1 (*7*−*10*, *12*, *13*, *16*−*18*).

Structurally, fumonisins are similar to sphingolipids and have been shown to inhibit sphingolipid biosynthesis via the ceramide synthase pathway (*19*). The consumption of contaminated maize-based foods is associated with a high incidence of esophageal and liver cancers, along with neural tube defects (*20*). Furthermore, outbreaks of leukoencephalomalacia in horses and pulmonary edema and hydrothorax in pigs have been linked to consumption of fumonisin-contaminated feed (*20*, *21*). The regulatory limit for fumonisins in maize is 2−4 μg/g total fumonisins (*22*) in the United States and 0.2−2 μg/g in the European Union (*23*). Thus, the findings of 1−36 mg/kg in selected highly infected single raisins and artificially contaminated raisins clearly exceeds regulatory limits (*13*, *14*) and are therefore of public health concern. However, to our knowledge, no studies have reported average fumonisin levels in commercial raisins.

Raisin production methods vary in different parts of the world, but mainly involve sun-drying, shade-drying, or mechanical drying (*24*). Production of dark-colored raisins in the United States, Chile, and South Africa is by direct sun-drying (*24*), which usually takes between 2 and 4 weeks to reach the commercial limit of > 85% dry matter (*24*, *25*). Faster drying may be obtained by the use of dipping solutions, commonly used in Mediterranean regions, where grapes are pretreated with solutions of 5−6% K_2CO_3 and olive oil to remove the waxy outer layer prior to sun-drying (*26*). This practice is generally used to produce the more light-colored sultana raisin variant (*24*−*26*). Golden variants are generally mechanically dried after pretreatment with SO_2 to retard nonenzymatic and enzymatic browning (*24*). Damaged grapes will likely become contaminated, especially by black aspergilli, during the drying period, because these fungi are present on both the grapes and the finished raisins (*13*). To keep

---

*Corresponding author (phone +45 45 25 26 02; fax + 45 45 88 41 48; e-mail kfn@bio.dtu.dk).



	M_r	R_1	R_2	R_3
FB_1	721	H	OH	OH
FB_2	705	H	OH	H
FB_3	705	H	H	OH
FB_4	689	H	H	H
FB_6	721	OH	OH	H

Figure 1. Structure of fumonisins B_1−B_4 and B_6.

ochratoxin A levels low, careful removal of infected raisins is enforced.

In this study, we investigated the presence of fumonisins in commercial raisins using cation-exchange purification with subsequent LC-MS/MS determination. We also investigated various extraction techniques to accommodate the high amounts of interfering material in raisins.

MATERIALS AND METHODS

All solvents were of HPLC grade, and other chemicals were of analytical grade. Reagents were from Sigma-Aldrich (St. Louis, MO). Water was purified on a Milli-Q system (Millipore, Bedford, MA). The fumonisin standard was a mixed certified standard containing fumonisins B_1 at 50.2 mg/L and B_2 at 51.0 mg/L (Biopure, Tulln, Austria). Fumonisin B_3 was from Biopure, and fumonisins B_4 and B_6 were from previous studies (*8*).

Raisin Samples. Raisins of 21 different brands were purchased from supermarkets and specialty shops in Denmark, Germany, and The Netherlands. The raisins were from the United States/California ($n = 9$), Greece ($n=1$), South Africa ($n=2$), Chile ($n=5$), Turkey ($n=3$), and China ($n = 1$). The 21 different brands represented a total of six different grape varieties including Thompson seedless, Jumbo Regal seedless, Flame, Sultana, Zante Currant, and Golden seedless.

Optimization of Sample Preparation. Raisins were homogenized with water (1:1 w/w) in a domestic kitchen blender. The mash was subsequently spiked with fumonisins and extracted. For method development, mixtures of water/methanol, water/ethanol, and water/acetonitrile, in 10% stepwise concentration increases from 30 to 100% organic solvent, were tested and the extracts purified using several formats (30 or 60 mg in 1 or 3 mL) of Strata X-C columns (Phenomenex, Torrance, CA). The effects of formic acid addition during extraction and different extraction volumes (3−20 times the amount of mash) were tested, along with different concentrations of NH_4OH for elution from the cation-exchange columns.

Sample Preparation. Raisins (≥200 g) were homogenized as described above, and 34 g of mash was transferred and split into two 50 mL Falcon tubes. The homogenates in each tube were extracted with 40 mL of acetonitrile/water/formic acid (86:12:2 v/v/v) under continuous shaking for 2 h. Samples were then centrifuged at 8000*g* for 5 min, and 35 mL of the upper phase of the resulting two-phase system was transferred to a clean 50 mL conical tube. Samples were evaporated to dryness under a N_2 flow, and each was redissolved in 10 mL of 1:1 v/v methanol/water, passed through a 0.45 μm PTFE syringe filter, and pooled. Sample purification was carried out using 60 mg/3 mL Strata X-C columns that had been preconditioned with 1 mL of methanol and 1 mL of water prior to sample load. To further acidify the column, 1 mL of water containing 2% formic acid was added, with a subsequent wash with 1 mL of methanol. Fumonisins were eluted with 1 mL of methanol/2% NH_4OH (1:4 v/v) into an autosampler vial and used directly for analysis.

LC-MS/MS conditions. LC-MS/MS analysis was performed as previously described (*11, 13*) on a Quattro Ultima triple mass spectrometer (Micromass, Manchester, U.K.) using positive electrospray ionization and operated in multiple-reaction mode (MRM or SRM) in the following transitions: fumonisins B_2 and B_3, quantifier m/z 706 → 336, cone, 50 V; collision, 40 eV; dwell time, 50 ms; qualifier m/z 706 → 318; cone 50 V; collision, 25 eV; dwell time, 100 ms; fumonisin B_4, quantifier m/z 690 → 320; cone, 50 V; collision, 35 eV; dwell time, 50 ms; qualifier m/z 690 → 338; cone, 50 V; collision, 30 eV; dwell time, 100 ms; fumonisins B_1 and B_6, quantifier m/z 722 → 334; cone, 50 V; collision, 40 eV; dwell time, 50 ms; qualifier m/z 722 → 528; cone, 50 V; collision, 25 eV; dwell time, 100 ms.

Calibration. Quantification was by external standard quantification using linear regression, with comparison to spiked raisin samples (raisins/water 1:1 w/w) incubated for 2 h prior to extraction. Samples were spiked with a certified mixture of fumonisins B_1 and B_2 to 0 (blank), 1, 5, 15, 50, or 100 μg/kg dry raisins. Preliminary results had indicated that the samples had low fumonisin levels that were close to the limit of quantitation (LOQ), so each homogenized sample was analyzed in triplicate. Because available amounts of fumonisins B_4 were low, its response factor relative to fumonisin B_2 was determined on the basis of pure standards.

Validation. Fumonisin B_2 determination was validated by spiking portions of 34 g of raisin mash (raisin/water 1:1 w/w) with a mixture of fumonisins B_1 and B_2 to concentrations of 0 (blank), 1, 5, 15, 50, or 100 μg/kg dry raisins. Experiments were performed on three different days, with all levels assayed in triplicate each day. Apparent recovery was calculated as the ratio of the slope of the spiked raisins to a standard dilution series in methanol/water (20:80 v/v) diluted to 0, 1, 5, 15, 50, and 100 μg/kg. Fumonisins B_6 and B_3 were occasionally coanalyzed in sequence to ensure sufficient separation.

RESULTS AND DISCUSSION

Sample Purification. Fumonisins have traditionally been purified using anion-exchange or immunoaffinity columns; however, in previous studies we observed that raisin extracts fouled immunoaffinity columns, probably due to polysaccharides and brown pigments (*13*), resulting in brownish viscous methanol eluates. Poor recovery has been observed when using anion-exchange columns for grape extracts and wine, most likely

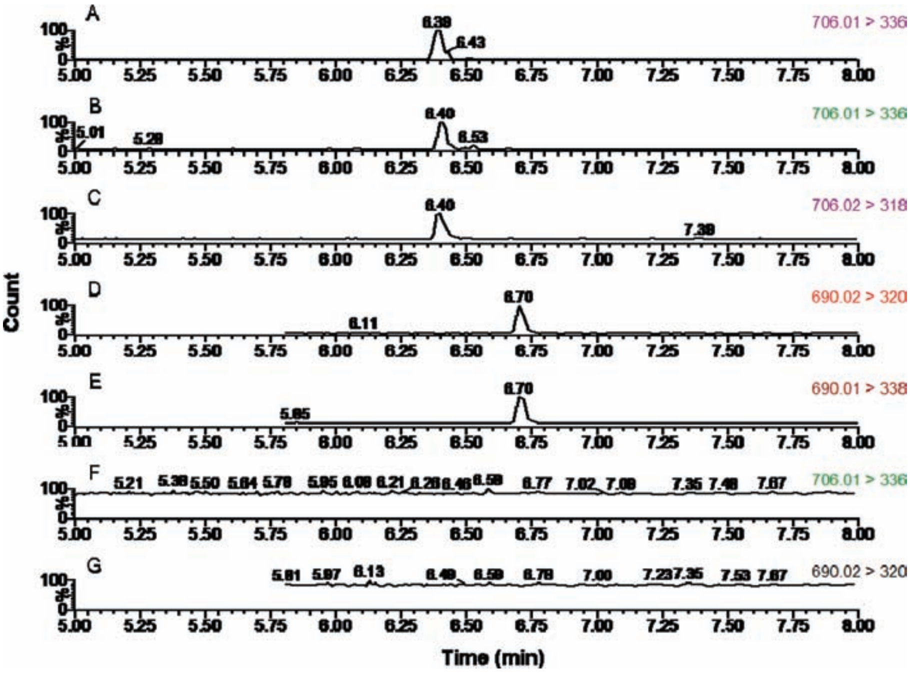


Figure 2. LC-MS/MS analysis; fumonisin B_2 and B_4 transitions in a fumonisin B_2-spiked sample and a blank raisin sample: (**A**) quantifier of fumonisin B_2 in a spiked raisin sample (5 μg/kg); (**B**) quantifier and (**C**) qualifier of fumonisin B_2 in a raisin sample (4.6 μg/kg); (**D**) quantifier and (**E**) qualifier of fumonisin B_4 in a raisin sample (1.2 μg/kg); (**F**) quantifier of fumonisin B_2 in a noncontaminated raisin sample; (**G**) quantifier of fumonisin B_4 in a noncontaminated sample.

because of the high content of organic acids, which salt-out fumonisins (*11*, *13*). To circumvent this problem, we used polymeric mixed-mode cation reversed phase columns, which target the amine of the fumonisins and have been proven effective for purifying fumonisins from wine (*11*).

During method development, we tested ethanol/water and methanol/water mixtures for extraction, because they did not result in a two-phase system as acetonitrile/water did, as also observed for aflatoxin extraction in various dry sample types (*27*). However, extensive amounts of pigments and polysaccharides (as judged from the extract viscosity) subsequently interfered with solid-phase extraction column function. Because this did not occur using acetonitrile extracts, we selected this QuEChERS-like method (*28*) in which the acetonitrile/water phase separates because of sugars from the raisins and performed further optimizations on this. Not surprisingly, the extracts needed to be acidified to keep the fumonisins in the acetonitrile phase. We also found that the volume of acetonitrile increased with decreasing temperature (observed when extracted stored in the refrigerator), so the samples were processed at the same temperature as the spiked samples used for calibration. We used two 50 mL conical tubes instead of a single 100 mL tube for extraction and extract evaporation to fit our centrifuge and N_2 evaporation equipment.

Validation. The LOQ was determined to be 1 μg/kg for fumonisin B_2 at a relative standard deviation of 35% ($n = 9$), which is highly satisfactory for this low level in this type of matrix. Trueness was 89, 101, and 97% for the three days, with average deviations of 22, 8, and 9%. The limit of detection (LOD, s/n 5) was at least 0.3 μg/kg for fumonisin B_2 and 0.3 and 0.1 μg/kg for fumonisin B_4. Apparent recovery was 87 ± 15%.

Screening of Raisin Samples. Our analysis demonstrated that 48% of the 21 tested raisin brands contained fumonisin B_2 and 43% fumonisin B_4. The detected amounts of fumonisins B_2 and B_4 in the positive samples ranged from the LOD (0.3 and 0.1 μg/kg) to 13 and 1.3 μg/kg, respectively. LC-MS/MS MRM chromatograms from selected spiked and nonspiked samples are shown in **Figure 2**.

The detection of fumonisin B_4 from retail raisins matches previous studies on artificially infected grapes and raisins (*13*); however, fumonisin B_4 was not identified in studies of fumonisin content in wine and grape must (*10*−*12*). This might suggest a different growth physiology and metabolism of *A. niger* on the drying grapes, possibly because of a fast fungal growth rate that results in some fumonisin B_4 not being transformed into B_2 before the water activity decreases below *A. niger* growth limits (∼0.85) (*4*). This postharvest growth along with the physical removal of water from the vine fruit and the concomitant concentration of the fumonisins may explain why fumonisin B_4 has not been detected in previous wine studies (*10*, *11*). The presence of fumonisin B_4 in raisins could also explain our higher frequency of positive samples because, in contrast to wine production, both the postharvest period and the drying are sources of fumonisin production and upconcentration.

The frequency of fumonisin contamination in wine is approximately 18−23% (*10*, *11*), which is half the frequency observed in the raisins in this study, fitting well with the higher frequency being caused by postharvest growth of *A. niger*. This correlates well with studies demonstrating a significant increase in fungal load during drying, exceeding the expected increase due to the concentration effect of water evaporation(*4*).

The levels we detected were > 1000-fold lower than in samples analyzed by Varga et al. (*14*), in which fumonisin B_1 was also detected. The finding of fumonisin B_1 is highly unlikely because bioinformatic analysis of the data from the three fully sequenced *A. niger* strain genomes (*15*) has not detected a *FUM2* gene encoding the enzyme responsible for C-10 hydroxylation (*29*). Thus, *A. niger* should not produce fumonisins B_1 and B_3 as also reported here and in all other studies analyzing extracts from *A. niger* strains (*7*−*10*, *12*, *13*, *16*−*18*).

Of the 10 positive samples in this study, 7 were derived from California ($n = 9$), 2 from Chile, and 1 from Turkey (**Table 1**), demonstrating a higher infection rate in the California raisins. The reasons for this are unknown and could be because of the different climate, preharvest weather conditions, different grape

Table 1. Overview of Tested Raisin Samples and Average Fumonisin Content[a]

brand	origin	grape variety	organic	fumonisin B$_2$ (μg/kg)	fumonisin B$_4$[b] (μg/kg)
1	California	Thompsons Seedless		4.9 ± 0.4	0.26 ± 0.05
1				4.5 ± 1	0.8 ± 0.4
1				13.0 ± 1	1.3 ± 0.4
1				7.5 ± 0.8	0.9 ± 0.2
2	California	Thompsons Seedless		2.7 ± 0.2	0.29 ± 0.1
3	California	Thompsons Seedless	√	1.8 ± 0.2	<LOQ[c]
4	California	Thompsons Seedless	√	2.6 ± 0.4	0.83 ± 0.5
5	California	Thompsons Seedless		3.8 ± 0.6	0.31 ± 0.09
6	California	Thompsons Seedless		4.1 ± 0.6	1.0 ± 0.1
7	California	Thompsons Seedless	√	1.3 ± 0.6	0.25 ± 0.2
8	California	Thompsons Seedless		ND[d]	ND
9	California	Thompsons Seedless	√	ND	ND
10	Turkey	Sultana	√	ND	ND
11	Turkey	Sultana	√	ND	ND
12	Turkey	Sultana		4.8 ± 2	0.75 ± 0.1
13	Greece	Zante currants		ND	ND
14	South Africa	Golden Seedless		ND	ND
15	South Africa	Jumbo Regal Seedless	√	ND	ND
16	China	unknown		ND	ND
17	Chile	Flame		ND	ND
18	Chile	Flame		ND	ND
19	Chile	Flame		ND	ND
20	Chile	Golden		<LOQ	ND
21	Chile	Golden		1.4 ± 0.6	<LOQ

[a] Fumonisin concentration is the average of three determinations from the same package, ±one standard deviation. [b] Fumonisin B$_4$ was calculated assuming a 4-fold better response factor than for fumonisin B$_2$ and is shown from one spiking experiment. Fumonisins B$_1$ and B$_6$ were not detected in any of the packages. [c] LOQ, limit of quantification: for fumonisin B$_2$, 1 μg/kg; for fumonisin B$_4$, estimated as 0.25 μg/kg. [d] ND, not detected. The limit of detection for fumonisin B$_2$ was 0.3 μg/kg, and that for fumonisin B$_4$ was 0.1 μg/kg.

cultivars, or different drying and sorting techniques (*4*). No connection was observed between fumonisin content and type of cultivation strategy (organic/conventional).

Large package variations were observed with up to 3-fold differences between four packages of the same brand, indicating a nonhomogeneous infection level, which may be due to contaminated raisins. Even though the detected fumonisin levels were 50−100 times below the regulatory limits of similar commodities such as maize, a larger survey is needed, both because of the low number of retail brands tested and because of the high variation among packages. Furthermore, establishing whether raisins may have even higher concentrations under different climatic conditions and drying procedures is important.

We presume that the low levels that we report here are also because of the efficient removal of damaged grapes that was initiated after problems with ochratoxin A in grapes and derived products were reported in the late 1990s (*30*−*32*), leading to very strict European Community regulations (472/2002) (*33*), including a maximum allowance of 10 μg/kg ochratoxin A in dried vine fruits (*34*).

In conclusion, this first survey on fumonisin determination in retail raisins demonstrated that almost 50% of tested brands contained low levels of fumonisins (1−14 μg/kg), which is far from the maximum allowable levels in regulated foods such as maize. Nonetheless, our finding of a high frequency of fumonisin occurrence clearly calls for larger surveys that can establish whether certain regions or weather conditions lead to fumonisin concentrations above set regulatory limits, as well as investigations of the influence of the drying process, especially on the production of fumonisin B$_4$.

LITERATURE CITED

(1) Schuster, E.; Dunn-Coleman, N.; Frisvad, J. C.; van Dijck, P. W. M. On the safety of *Aspergillus niger* − a review. *Appl. Microbiol. Biotechnol.* **2002**, *59*, 426−435.

(2) Zahavi, T.; Cohen, L.; Weiss, B.; Schena, L.; Daus, A.; Kaplunov, T.; Zutkhi, J.; Ben-Arie, R.; Droby, S. Biological control of *Botrytis*, *Aspergillus* and *Rhizopus* rots on table and wine grapes in Israel. *Postharvest Biol. Technol.* **2000**, *20*, 115−124.

(3) Battilani, P.; Giorni, P.; Pietri, A. Epidemiology of toxin-producing fungi and ochratoxin A occurrence in grape. *Eur. J. Plant Pathol.* **2003**, *109*, 715−722.

(4) Leong, S. L.; Hocking, A. D.; Pitt, J. I. Occurrence of fruit rot fungi (*Aspergillus* section Nigri) on some drying varieties of irrigated grapes. *Aust. J. Grape Wine Res.* **2004**, *10*, 83−88.

(5) Magnoli, C.; Violante, M.; Combina, M.; Palacio, G.; Dalcero, A. Mycoflora and ochratoxin-producing strains of *Aspergillus* section *Nigri* in wine grapes in Argentina. *Lett. Appl. Microbiol.* **2003**, *37*, 179−184.

(6) Da Rocha Rosa, C. A. D.; Palacios, V.; Combina, M.; Fraga, M. E.; Rekson, A. D.; Magnoli, C. E.; Dalcero, A. M. Potential ochratoxin A producers from wine grapes in Argentina and Brazil. *Food Addit. Contam.* **2002**, *19*, 408−414.

(7) Frisvad, J. C.; Smedsgaard, J.; Samson, R. A.; Larsen, T. O.; Thrane, U. Fumonisin B$_2$ production by *Aspergillus niger*. *J. Agric. Food Chem.* **2007**, *55*, 9727−9732.

(8) Månsson, M.; Klejnstrup, M. L.; Phipps, R. K.; Nielsen, K. F.; Frisvad, J. C.; Gotfredsen, C. H.; Larsen, T. O. Isolation and NMR characterization of fumonisin B$_2$ and a new fumonisin B$_6$ from *Aspergillus niger*. *J. Agric. Food Chem.* **2010**, *58*, 949−953.

(9) Noonim, P.; Mahakarnchanakul, W.; Nielsen, K. F.; Frisvad, J. C.; Samson, R. A. Fumonisin B$_2$ production by *Aspergillus* niger in Thai coffee beans. *Food Addit. Contam. Part A: Chem. Anal. Control Expo. Risk Assess.* **2009**, *26*, 94−100.

(10) Logrieco, A.; Ferracane, R.; Visconti, A.; Ritieni, A. Natural occurrence of fumonisin B$_2$ in red wine from Italy. *Food Addit. Contam. Part A: Chem. Anal. Control Expo. Risk Assess.* **2010**, *27*, 1136−1141.

(11) Mogensen, J. M.; Larsen, T. O.; Nielsen, K. F. Widespread occurrence of the mycotoxin fumonisin B$_2$ in wine. *J. Agric. Food Chem.* **2010**, *58*, 4853−4857.

(12) Logrieco, A.; Ferracane, R.; Haidukowsky, M.; Cozzi, G.; Visconti, A.; Ritieni, A. Fumonisin B$_2$ production by *Aspergillus niger* from grapes and natural occurrence in must. *Food Addit. Contam. Part A: Chem. Anal. Control Expo. Risk Assess.* **2009**, *26*, 1495−1500.

(13) Mogensen, J. M.; Frisvad, J. C.; Thrane, U.; Nielsen, K. F. Production of fumonisin B$_2$ and B$_4$ by *Aspergillus niger* on grapes and raisins. *J. Agric. Food Chem.* **2010**, *58*, 954−958.

(14) Varga, J.; Kocsube, S.; Suri, K.; Szigeti, G.; Szekeres, A.; Varga, M.; Toth, B.; Bartok, T. Fumonisin contamination and fumonisin producing black aspergilli in dried vine fruits of different origin. *Int. J. Food Microbiol.* **2010**, *143*, 143−149.

(15) Pel, H. J.; de Winde, J. H.; Archer, D. B.; Dyer, P. S.; Hofmann, G.; Schaap, P. J.; Turner, G.; de Vries, R. P.; Albang, R.; Albermann, K.; Andersen, M. R.; Bendtsen, J. D.; Benen, J. A. E.; van den Berg, M.; Breestraat, S.; Caddick, M. X.; Contreras, R.; Cornell, M.; Coutinho, P. M.; Danchin, E. G. J.; Debets, A. J. M.; Dekker, P.; van Dijck, P. W. M.; van Dijk, A.; Dijkhuizen, L.; Driessen, A. J. M.; d'Enfert, C.; Geysens, S.; Goosen, C.; Groot, G. S. P.; de Groot, P. W. J.; Guillemette, T.; Henrissat, B.; Herweijer, M.; van den Hombergh, J. P. T. W.; van den Hondel, C. A. M. J.; van der Heijden, R. T. J. M.; van der Kaaij, R. M.; Klis, F. M.; Kools, H. J.; Kubicek, C. P.; van Kuyk, P. A.; Lauber, J.; Lu, X.; van der Maarel, M. J. E. C.; Meulenberg, R.; Menke, H.; Mortimer, M. A.; Nielsen, J.; Oliver, S. G.; Olsthoorn, M.; Pal, K.; van Peij, N. N. M. E.; Ram, A. F. J.; Rinas, U.; Roubos, J. A.; Sagt, C. M. J.; Schmoll, M.; Sun, J. B.; Ussery, D.; Varga, J.; Vervecken, W.; de Vondervoort, P. J. J. V.; Wedler, H.; Wosten, H. A. B.; Zeng, A. P.; van Ooyen, A. J. J.; Visser, J.; Stam, H. Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. *Nat. Biotechnol.* **2007**, *25*, 221−231.

(16) Susca, A.; Proctor, R. H.; Mule, G.; Stea, G.; Ritieni, A.; Logrieco, A.; Moretti, A. Correlation of mycotoxin fumonisin B_2 production and presence of the fumonisin biosynthetic gene fum8 in *Aspergillus niger* from grape. *J. Agric. Food Chem.* **2010**, *58*, 9266−9272.

(17) Mogensen, J. M.; Nielsen, K. F.; Frisvad, J. C.; Thrane, U. Effect of temperature and water activity on the production of fumonisins by *Aspergillus niger* and different *Fusarium* species. *BMC Microbiol.* **2009**, *9*, 281.

(18) Nielsen, K. F.; Mogensen, J. M.; Johansen, M.; Larsen, T. O.; Frisvad, J. C. Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. *Anal. Bioanal. Chem.* **2009**, *395*, 1225−1242.

(19) Marasas, W. F.; Riley, R. T.; Hendricks, K. A.; Stevens, V. L.; Sadler, T. W.; Gelineau-van, W. J.; Missmer, S. A.; Cabrera, J.; Torres, O.; Gelderblom, W. C.; Allegood, J.; Martinez, C.; Maddox, J.; Miller, J. D.; Starr, L.; Sullards, M. C.; Roman, A. V.; Voss, K. A.; Wang, E.; Merrill, A. H., Jr. Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. *J. Nutr.* **2004**, *134*, 711−716.

(20) Stockmann-Juvala, H.; Savolainen, K. A review of the toxic effects and mechanisms of action of fumonisin B1. *Hum. Exp. Toxicol.* **2008**, *27*, 799−809.

(21) Yazar, S.; Omurtag, G. Z. Fumonisins, trichothecenes and zearalenone in cereals. *Int. J. Mol. Sci.* **2008**, *9*, 2062−2090.

(22) U.S. Food and Drug Administration. *Guidance for Industry: Fumonisin Levels in Human Foods and Animal Feeds*; U.S. Food and Drug Administration: Washington, DC, 2001.

(23) EC 1126/2007 amending Regulation No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards *Fusarium* toxins in maize and maize products. *Commission Eur. Communities* **2007**, *1126*, 14−16.

(24) Esmaiili, M.; Sotudeh-Gharebagh, R.; Cronin, K.; Mousavi, M. A. E.; Rezazadeh, G. Grape drying: a review. *Food Rev. Int.* **2007**, *23*, 257−280.

(25) Dincer, I. Sun drying of Sultana grapes. *Dry. Technol.* **1996**, *14*, 1827−1838.

(26) Doymaz, I.; Pala, M. The effects of dipping pretreatments on air-drying rates of the seedless grapes. *J. Food Eng.* **2002**, *52*, 413−417.

(27) Stroka, J.; Petz, M.; Joerissen, U.; Anklam, E. Investigation of various extractants for the analysis of aflatoxin B-1 in different food and feed matrices. *Food Addit. Contam.* **1999**, *16*, 331−338.

(28) Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412−431.

(29) Butchko, R. A. E.; Plattner, R. D.; Proctor, R. H. Deletion analysis of FUM genes involved in tricarballylic ester formation during fumonisin biosynthesis. *J. Agric. Food Chem.* **2006**, *54*, 9398−9404.

(30) Majerus, P.; Otteneder, H. Detection and occurrence of ochratoxin A in wine and grapejuice. *Dtsch. Lebensm.-Rundsch.* **1996**, *92*, 388−390.

(31) Zimmerli, B.; Dick, R. Ochratoxin A in table wine and grape-juice: occurrence and risk assessment. *Food Addit. Contam.* **1996**, *13*, 655−668.

(32) Burdaspal, P.; Legarda, T. Ochratoxin A in wines and grape products originated from Spain and other european countries. *Alimentaria* **1999**, *36*, 107−113.

(33) EC 1881/2006. Setting maximum levels for certain contaminants in foodstuffs. *Commission Eur. Communities* **2006**, *472*, 18−20.

(34) EC 472/2002. Setting maximum levels for certain contaminants in foodstuffs. *Commission Eur. Communities* **2002**, *472*, 18−20.

Received for review October 3, 2010. Revised manuscript received November 21, 2010. Accepted November 29, 2010. J.M.M., P.B.K., and K.F.N. were funded by the Danish Food Industry Agency (Grant 3304-FVEP-07-730-01). The EEC project MycoRed (KBBE-2007-222690-2) is acknowledged for financial support.