JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Evaluation of Conventional and Organic Italian Foodstuffs for Deoxynivalenol and Fumonisins B_1 and B_2

TERESA CIRILLO,* ALBERTO RITIENI, MARIANNA VISONE, AND RENATA AMODIO COCCHIERI

Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli, Federico II, Via Università 100, 80055 Portici, Napoli, Italy

Two lots of human foodstuffs from conventional and organic brand foods were purchased from supermarkets and analyzed for three *Fusarium* toxins, deoxynivalenol, by GC-ECD, and fumonisins B_1 and B_2 (FB_1-FB_2), by LC-MS. The occurrence of deoxynivalenol contamination was higher than 80% in both organic and conventional foods; fumonisin B_1 was found in 20% of organic foods and in 31% of conventional ones and fumonisin B_2 in more than the 32% of the food samples from both the agricultural practices. The highest median concentration of deoxynivalenol occurred in conventional rice-based foodstuffs (207 μg/kg): that of fumonisin B_1 in conventional maize-based foods (345 μg/kg) and that of fumonisin B_2 in organic wheat-based foods (210 μg/kg).

KEYWORDS: Deoxynivalenol; fumonisins; human foods; conventional and organic agriculture

INTRODUCTION

During recent years, organic agricultural practices have grown considerably as an alternative to conventional agriculture and related use of chemical pesticides. Consumers expect that the organic agriculture has benefits with respect to food safety and flavor of fruits and vegetables. However, pesticide and chemical residues are only two of the issues related to contamination of the food chain. Another risk for human health is due to mold infection that can affect conventional as well as organic cereals, because fungi can produce bioactive mycotoxins. When introduced to higher vertebrates by a natural route even in low concentrations, these metabolites may produce toxic effects (*1*, *2*). Among fungi, *Fusarium* species are considered worldwide the main threat for cereals that represent the most important source of human food (*3*). These fungi can produce several mycotoxins such as trichothecenes and fumonisins. The most important trichothecene is deoxynivalenol also known as vomitoxin. Deoxynivalenol has been detected in several types of grain and cereals throughout the world, including industrialized countries such as Canada, England, United States, and Finland, at levels ranging from submicrograms per kilogram to milligrams per kilogram levels (*4*−*9*). Deoxynivalenol occurrence is enhanced during harvest seasons characterized by high humidity.

Fumonisins are very often present in maize products and fumonisin B_1 is considered by IARC a "possibly carcinogenic to humans" (Group 2B) (*10*). Deoxynivalenol and fumonisins are thermostable requiring for degradation $T > 210$ °C for 40 min and $T > 220$ °C for 25 min, respectively (*4*, *11*, *12*).

* To whom correspondence should be addressed. Phone: +39-81-2539410. Fax: +39-81-2539407. E-mail: tcirillo@unina.it.

The objective of this study was to evaluate and compare the occurrence of deoxynivalenol, fumonisin B_1, and fumonisin B_2 in traditional and organic brand foods purchased in Italy.

MATERIALS AND METHODS

Conventional and organic foodstuff specimens were obtained from supermarkets in the Campania region (Italy). For each batch two commercial packages were collected that were analyzed separately; a total of 404 analytical samples were examined. The mycotoxin contents were calculated as average of the results obtained by all the determinations.

Maize-based foodstuff consisted of popcorn, flour, couscous, polenta, biscuits, and breakfast products: 27 batches each of conventional and organic products.

Wheat-based foodstuff consisted of raw material, flour, bran, biscuits, bread, and pasta: 44 batches of conventional and 36 of organic products.

Rice-based foodstuff consisted of raw material, biscuits, and rice flakes: 13 batches of conventional products and 11 of organic products.

Mixed-based foodstuff consisted of rye, barley, spelt, millet, oats milled cereals, wholemeal, soups, and breakfast cereals: 17 batches of conventional products and 29 of organic products.

The total collected samples included 101 batches of conventional and 101 of organic products.

Each commercial package was completely ground by a HMHF Turbo Homogenizer high-speed blade homogenizer (PBI International, Milano, Italy) for 3 min, subdivided into aliquots of 15 and 10 g to quantify deoxynivalenol and fumonisins, respectively, and stored at −20 °C until analyses.

Analytical Reagents. Analytical grade reagents and GC and HPLC grade solvents were purchased from Merck KgaA (Darmstadt, Germany). Deoxynivalenol and fumonisins B_1 and B_2 standards were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, Missouri).

Deoxynivalenol Determination. Deoxynivalenol was determined according to the Tacke and Casper (*13*) method. Aliquots of 15 g of each sample were extracted by stirring twice at 200 rpm with

10.1021/jf030203h CCC: $25.00
Published on Web 11/25/2003

Table 1. Contamination Levels (median and range in μg/kg) of Deoxynivalenol and Fumonisins B1 and B2 in the Cereal-Based Conventional and Organic Foodstuffs Analyzed

foodstuffs analyzed	mycotoxins	conventional foods		organic foods	
		median	range	median	range
total	**deoxynivalenol**	**65**	**7–450**	**65**	**9–930**
	fumonisin B1	**80**	**20–2870**	**67**	**10–1970**
	fumonisin B2	**90**	**10–400**	**150**	**30–790**
maize based	deoxynivalenol	65	14–450	65	19–800
	fumonisin B1	345	27–2160	185	10–600
	fumonisin B2[a]	20	10–400	120	30–150
wheat based	deoxynivalenol[a]	65	7–310	38	9–210
	fumonisin B1[a]	97	40–2870	25	15–67
	fumonisin B2	90	10–380	210	70–790
rice based	deoxynivalenol[a]	207	12–440	65	25–510
	fumonisin B1[a]	30	20–80	145	20–350
	fumonisin B2[a]	205	10–400	150	140–380
mixed based	deoxynivalenol	65	12–89	65	65–930
	fumonisin B1	70	60–420	50	10–1970
	fumonisin B2	65	10–70	60	35–140

[a] $p < 0.05$.

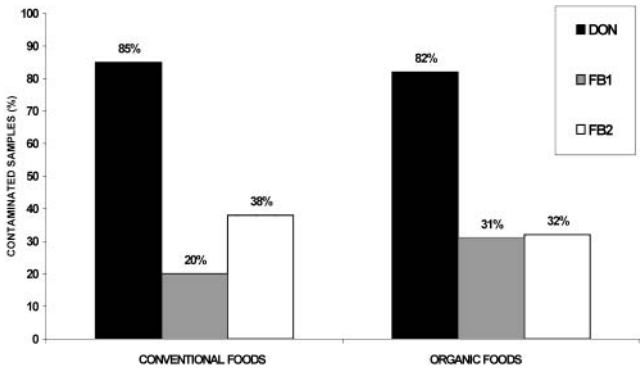

Figure 1. Occurrence of deoxynivalenol and fumonisins B1 and B2 in all the samples of conventional and organic Italian marketed foodstuffs analyzed.

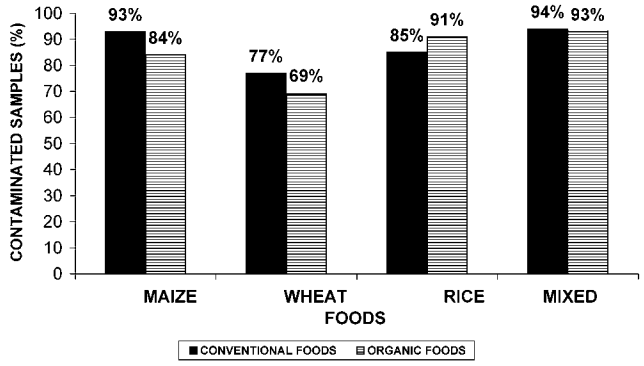

Figure 2. Occurrence of deoxynivalenol in the different conventional and organic cereal-based foodstuffs analyzed.

acetonitrile−water (84:16 v/v) (40 mL and then 30 mL) for 60 min at room temperature. The extracts were partially concentrated under vacuum at 40 °C and reconstituted to 40 mL with acetonitrile−water (84:16 v/v); 10% of this solution was purified. Cleanup columns, 7 cm × 1 cm, were prepared from 1 g of a mixture of 50 g of silica gel (0.063−0.200 mm) and 150 g of aluminum oxide 90 active neutral (70−230 mesh, 0.063−0.200 mm), activated for 12 h at 60 °C and stored until deoxynivalenol purification in a dry atmosphere.

Two 4-mL aliquots of each extract were loaded into the dry cleanup column and eluted by gravity, obtaining two eluates that were separately dried under nitrogen flow.

Deoxynivalenol detection was carried out by GC-ECD analysis of two different derivatives.

The first was obtained by reacting the sample with trimethylsilylimidazole−trimethylchlorosilane (TMSI−TMCS) (Merck KgaA, Darmstadt, Germany) (*13*), while for the second one the sample was reacted with trifluoracetic acid anhydride (TFAA) (Serva, U.S.A.) (*14*).

TMSI−TMCS Derivatization. A 100-μL sample of TMSI−TMCS (100:1) was loaded into a vial containing the dried eluate with continued stirring for 10 min at room temperature. The derivatized sample was diluted with 1 mL of a 0.125 μg/mL solution of Mirex (Dechlorane) (Superchrom s.r.l., Italy), as internal standard, in 2,2,4-trimethylpentane solution and with 1 mL of water. The clear upper organic layer was transferred in a vial for the gas chromatographic analysis.

TFAA Derivatization. Confirmation tests were carried out by TFAA derivatization as follows: A 100-μL sample of TFAA and 10 mg of $NaHCO_3$ were added to the dried eluate and the vial was heated for 20 min at 60 °C. The excess of TFAA reagent was removed by a gentle flow of nitrogen, and 300 μL of toluene and 1 mL of water were added, shaking the mixture for 30 s. The upper toluene layer was collected and dried with 10 mg of anhydrous sodium sulfate. A 200-μL aliquot was placed into a vial containing 50 μL of a PCB 138 (Dr. Ehrenstorfer, GmbH Germany) solution, 0.5 μg/mL in toluene, and analyzed by GC-ECD.

Deoxynivalenol analyses were performed on a Hewlett-Packard 5890 Series II (U.S.A.) gas chromatograph, with electron capture detector. A 30 m × 0.32 mm i.d., 0.25 μm, HP-5 capillary column was used (J&W Scientific, Folsom, CA).

The conditions used to detect the TMSI−TMCS derivates were the following: injection port temperature 300 °C; mode of injection splitless and injection volume 1 μL. The carrier gas was helium at a flow of 1.8 mL/min and the makeup gas was a mix of argon and methane (95:5) at a flow of 30.5 mL/min. The ECD temperature was fixed at 320 °C. The initial oven temperature was 70 °C, which was then increased to 170 °C at 25 deg/min and to 300 °C at 5 deg/min and held for 2 min.

The GC detection of the TFFA derivates was performed by using nitrogen as the carrier gas at a constant flow of 40 mL/min; the injection volume was 1 μL, the mode was splitless, the period was 2 min, the injector temperature was 225 °C, and the detector temperature was 300 °C. The temperature program was as follows: initial temperature was 80 °C for 2 min, then increasing 20 deg/min to 175 °C, 1 deg/min to 245 °C, and 10 deg/min to 270 °C, where it was held for 10 min.

The limit of quantitation for both methods was 7 μg/kg.

Recovery of deoxynivalenol was calculated at three different spiking levels, 50, 100, and 200 μg/kg, and each test was replicated twice. The average recovery calculated was 91 ± 5%.

Fumonisin B_1 and B_2 Determination. The extraction of fumonisins B_1 and B_2 was obtained by homogenizing every ground product (10 g) for 3 min in methanol (50 mL) with an Ultraturrax T 25 Basic (IKA Labortechnik, Germany). The liquid phase was centrifuged 15 min at 3000 rpm and then 25 mL was dried under vacuum at 40 °C. The dry

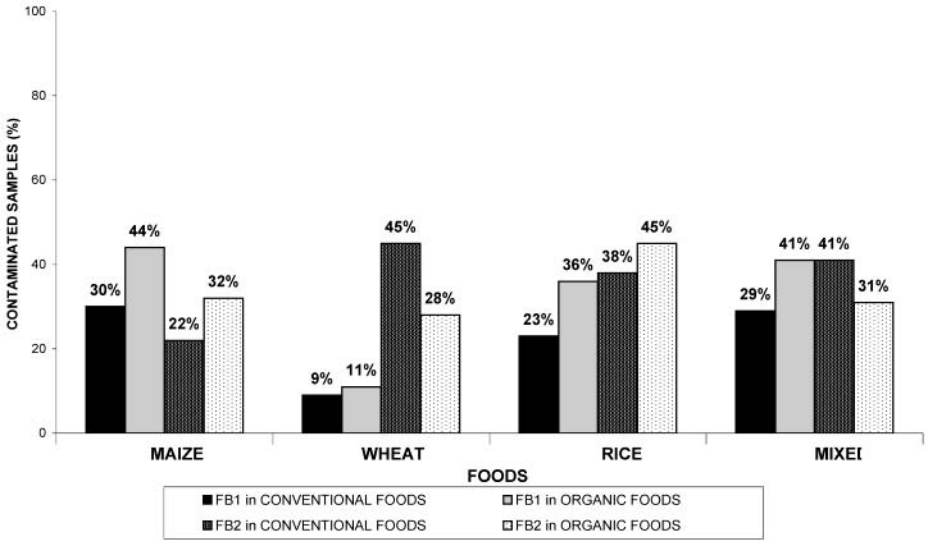

Figure 3. Occurrence of fumonisins B1 and B2 in the different conventional and organic cereal-based foodstuffs analyzed.

extract was reconstituted with methanol (5 mL) and filtered through a 0.22-μm membrane, and 20 μL was injected onto the HPLC column.

Fumonisin LC/MS Analysis. For the fumonisin detection an API 100 LC single-quadrupole mass spectrometer (Perkin-Elmer Corporation, Norwalk, CT) with electrospray ion source in the positive ion mode was used. A flow rate of 30 μL/min was split from the LC eluent into the ion spray source. A probe voltage of 5300 V and declustering potential of 50 V were used. A 20-μL sample was introduced by a Rheodyne external loop injector onto a 33 $\times$ 4.6 mm^2 i.d., 3.0 μm Pecosphere C_{18} (Brownlee Columns, Perkin-Elmer Corporation, Norwalk, CT). HPLC conditions were the following: solvent gradient with 0.1% trifluoroacetic acid in H_2O (A) and methanol/5 mM ammonium acetate (80:20 v/v) with 1% formic acid (B) changing from 70% to 100% of (B) during 13 min. A preliminary scan spectrum of pure fumonisin B_1 and B_2 standards was investigated to select ion peaks to quantify fumonisins. Blank matrix extracts were investigated to confirm that no spectrometric interferences came from the matrix. The spectrometric quantitative analysis was operated in the Single Ion Monitoring (SIM) mode with a dwell time of 300 m/s, using *m*/*z* 722.4 and 745.5 corresponding to sodium adduct, for fumonisin B_1 analysis and *m*/*z* 706.4 and 728.5 to detect fumonisin B_2 molecular ion peak and sodium adduct, respectively. This pair of ions for each fumonisin, combined with reproducible retention time recorded in all experiments, enabled the identity of the toxins to be confirmed. The method was linear for both fumonisins in the range 0.05−10.00 μg/mL and the limit of quantitation was 5 μg/kg for both fumonisins.

Glass and reagent blanks were periodically run to ensure no cross contamination. The average recoveries of fumonisins, calculated at three different spiking levels (50, 100, and 200 μg/kg), replicating each test twice, were 75 $\pm$ 8% for fumonisin B_1 and 68 $\pm$ 5% for fumonisin B_2.

Statistical Analysis. T-Test and variance analysis were performed by SPSS Base (SPSS Inc Chicago, Illinois, U.S.A.). No detectable samples were considered contaminated at the above-reported limits of detection of each analytical method applied.

RESULTS

The occurrence of contamination by deoxynivalenol and fumonisins B_1 and B_2 in analyzed foodstuffs is represented in **Figures 1**, **2**, and **3**. Contamination levels of deoxynivalenol and fumonisins B_1 and B_2, corrected for recoveries, are shown in **Table 1**, as medians and ranges.

Comparing all conventional and organic foods (**Figure 1**), deoxynivalenol occurrence was slightly more frequent in conventional food: 85% positive samples against 82%. For fumonisin occurrence, 20% of the conventional and 31% of the organic samples were contaminated by fumonisin B_1, while fumonisin B_2 was detected in 38% of the conventional samples and in the 32% of the organics.

Among the different cereal-based foodstuffs analyzed, the occurrence of deoxynivalenol contamination was higher in conventional than in organic foods, except for the rice-based products (**Figure 2**).

The occurrence of fumonisin B_1 contamination varied from 9% (conventional wheat products) to 30% (conventional maize products) and from 11% (organic wheat products) to 41% (mixed organic products). Organic maize and rice were more frequently contaminated by FB_2 than the corresponding conventional foods (**Figure 3**).

The deoxynivalenol contamination levels varied from 7 to 450 μg/kg in conventional foods and from 9 to 930 μg/kg in the organic ones, with median values of 65 μg/kg (**Table 1**). The highest level of deoxynivalenol (930 μg/kg) was found among the organic foods in a cereal-mixed soup. Deoxynivalenol median levels in conventional wheat and rice were significantly higher than those in the corresponding organic samples ($p < 0.05$), and the levels of fumonisin B_1 were significantly higher in conventional wheat-based foodstuffs than in the corresponding organics ($p < 0.05$). The conventional rice-based samples showed fumonisin B_2 median concentrations significantly higher than those found in the organic foods. Differences in deoxynivalenol and fumonisins B_1 and B_2 contamination levels among the other groups were not significant.

DISCUSSION

The range in the occurrence of the investigated mycotoxins is considerable, both in conventional and in organic foodstuffs. The frequency of contamination by deoxynivalenol appears much higher than that of fumonisins B_1 and B_2. Deoxynivalenol contamination was only a little higher in conventional than in organic foods. In rice-based foods, on the contrary, it was more frequent in organic foods but the calculated median level was about three times higher in conventional than in organic foods (207 against 65 μg/kg). According to previous data (*15*, *16*) maize-based foods are the most contaminated by fumonisin B_1. Rice should be further investigated for mycotoxins considering its high worldwide consumption levels. In Italy, also, rice consumers are increasing, daily consumers amounting to 53.4% of Italians (*17*). In addition, rice is often used as an ingredient in many food products such as chocolate bars, snacks, and other

foods largely consumed by children. Deoxynivalenol and fumonisins B_1 and B_2 were less frequently present in conventional rice food than in organic. Finally, considering mixed foods, the median levels of the mycotoxins appeared quite similar between organic and conventional products.

The results obtained in this study are comparable to the data shown by Schollenberger et al. (*16*) and by Malmauret et al. (*18*). The frequency of deoxynivalenol contamination in conventional German foods is lower than that found in the analogous Italian samples (44% versus 85%), while the levels of the median values appear very similar (62 and 65 μg/kg for German and Italian, respectively). On the other hand, the organic German foods were more frequently and severely contaminated than the Italian ones (86% at 78 μg/kg versus 82% at 65 μg/kg, respectively). Deoxynivalenol contamination in French foods shows lower median values in conventional wheat and barley samples (55 and 41 μg/kg) versus organic (106 and 69 μg/kg).

Deoxynivalenol levels detected in this study were lower than those found in the U.S.A. (*19*, *20*) in wheat, rice, and mixed food; deoxynivalenol levels in maize in Italy appear lower than those found in Indonesia (*21*). Conventional maize foods showed a contamination with reduction with respect to the 1994 Italian results (*22*) and were also lower with respect to English and Spanish data (*15*, *23*), an encouraging fact for the Italian market.

The results of this study demonstrate that a conclusion about differences in the quality of organic and conventional food requires further study and effort, particularly in a field scarcely investigated such as that related to mycotoxin contamination of the above-mentioned products.

LITERATURE CITED

(1) Bennet, J. W. Mycotoxins, mycotoxicoses, mycotoxicology and mycopathologia. *Mycopathologia*. **1987**, *100*, 3−5.

(2) Malloy, C. D.; Marr, J. S. Mycotoxins and public health: A review. *J. Public Health Manage Pract.* **1997**, *3*, 61−69.

(3) FAO Food and Agriculture Organization of the United Nations. The climate change-agriculture conundrum. 2001; data available on http://www.fao.org/docrep/W5183E/w5183e03.htm.

(4) Scott, P. M. Trichothecenes in grains. *Cereal Foods World* **1990**, *35*, 661−666.

(5) Beardall, J.; Miller, J. D. Natural occurrence of mycotoxin other than aflatoxin in Africa, Asia and South America. *Mycotoxin Res.* **1994**, *10*, 21−40.

(6) Doko, M. B.; Canet, C.; Brown, N.; Sydenham, E. W.; Mpuchane, S.; Siame, B. A. Natural co-occurrence of fumonisins and zearalenone in cereals and cereal-based foods from Eastern and Southern Africa. *J. Agric. Food Chem.* **1996**, *44*, 3240−3243.

(7) D'Mello, J. P. F.; Porter, J. K.; Macdonald, A. M. C.; Placinta, C. M. *Fusarium* mycotoxins. In *Handbook of Plant and Fungal Toxicants*; D'Mello, J. F. P., Ed.; CRC Press: Boca Raton, FL, 1997; pp 287−301.

(8) Desjardins, A. E.; Manandhar, H. K.; Plattner, R. D.; Manandhar, G. G.; Poling, S. M.; Maragos, C. M. *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. *Appl. Environ. Microbiol.* **2000**, *66*, 1020−1025.

(9) Desjardins, A. E.; Manandhar, G. G.; Plattner, R. D.; Maragos, C. M.; Shrestha, K.; McCormick, S. P. Occurrence of *Fusarium* species and mycotoxins in Nepalese maize and wheat and the effect of traditional processing methods on mycotoxin levels. *J. Agric. Food Chem.* **2000**, *48*, 1377−1383.

(10) IARC **2002**, *82*, 301−302, Data available on http://www.iarc.fr.

(11) Nowicky, T. W.; Gaba, D. G.; Dexter, J. E.; Matsuo, R. R.; Clear, R. M. Retention of the *Fusarium* mycotoxin deoxynivalenol in wheat during processing and cooking of spaghetti and noodles. *J. Cereal Sci.* **1988**, *8*, 189−199.

(12) Scott, P. M.; Lawrence, G. A. Stability and problems in recovery of fumonisins added to corn-based foods. *J. AOAC Int.* **1994**, *77*, 541−545.

(13) Tacke, B. K.; Casper, H. H. Determination of deoxynivalenol in wheat, barley and malt by column cleanup and gas chromatography with electron capture detection. *J. AOAC Int.* **1996**, *79*, 472−475.

(14) Kotal, F.; Holadovà, K.; Hajslovà, J.; Poustka, J.; Radovà, Z. Determination of trichothecenes in cereals. *J. Chromatogr. A* **1999**, *830*, 219−225.

(15) Patel, S.; Hazel, C. M.; Winterton, A. G. M.; Gleadle, A. E. Surveillance of fumonisins in UK maize-based foods and other cereals. *Food Addit. Contam.* **1997**, *14*, 187−191.

(16) Schollenberger, M.; Suchy, S.; Jara, H. T.; Drochner, W.; Muller, H. M. A survey of *Fusarium* toxins in cereal based foods marketed in an area of South-East Germany. *Mycopathologia* **1999**, *147*, 49−57.

(17) Turrini, A.; Saba, A.; Perrone, D.; Cialfa, E.; D'Amicis, A. Original communication food consumption patterns in Italy: The INN-CA Study 1994−1996. *Eur. J. Clin. Nutr.* **2001**, *55*, 571−588.

(18) Malmauret, L.; Parent-Massin, D.; Hardy, J. L.; Verger, P. Contaminants in organic and conventional foodstuffs in France. *Food Addit. Contam.* **2002**, *19*, 524−532.

(19) Trucksess, M. W.; Ready, D. E.; Pender, M. K. Determination and survey of deoxynivalenol in white flour, whole wheat flour and brain. *J. AOAC Int.* **1996**, *79*, 883−887.

(20) Trucksess, M. W.; Thomas, F.; Young, K.; Stack, M. E.; Fulguers, W. J.; Page, S. W. Survey of deoxynivalenol in US 1993 wheat and barley crop by enzyme-linked-immunosorbment-assay. *J. AOAC Int.* **1995**, *78*, 631−636.

(21) Ali, N.; Sardjono, T.; Yamashita, A.; Yoshizawa T. Natural co-occurrence of aflatoxins and *Fusarium* mycotoxins (fumonisins, deoxynivalenol, nivalenol, zearalenone) in corn from Indonesia. *Food Addit. Contam.* **1988**, *15*, 377−384.

(22) Doko, M. B.; Visconti, A. Occurrence of fumonisins B1 and B2 in corn and corn-based human foodstuffs in Italy. *Food Addit. Contam.* **1994**, *11*, 433−439.

(23) Castellà, G.; Bragulat, M. R.; Cabanes, J. Surveillance of fumonisins in maize-based feeds and cereals from Spain. *J. Agric. Food Chem.* **1999**, *47*, 4707−4710.

Received for review March 19, 2003. Revised manuscript received October 15, 2003. Accepted October 16, 2003.

JF030203H