JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Confirmation of Reduced Toxicity of Deoxynivalenol in Extrusion-Processed Corn Grits by the MTT Bioassay

YUKSEL CETIN AND LLOYD B. BULLERMAN*

Department of Food Science and Technology, 143 Filley Hall, University of Nebraska−Lincoln, Lincoln, Nebraska 68583-0919

The objective of this study was to determine the loss of toxicity of deoxynivalenol in extruded cereal-based products by the tetrazolium salt (MTT) bioassay using a sensitive Chinese hamster ovary (CHO-K1) cell line and to compare the results to chemical (high-performance liquid chromatography, HPLC) and biochemical (enzyme-linked immunosorbant assay, ELISA) methods of analysis. A split−split plot design was used for the extrusion process experiments at temperatures of 150, 175, and 200 °C and screw speeds of 70 and 140 rpm. The initial mean deoxynivalenol concentration in the corn grits artificially contaminated with *Fusarium graminearum* was found to be 23.5 μg/g as measured by HPLC. The percent reductions of deoxynivalenol in the contaminated corn grits upon extrusion processing ranged from 22 to 35%, from 21 to 34%, and from 21 to 37% as measured by HPLC, ELISA, and MTT bioassay, respectively. The MTT bioassay results were more closely correlated with HPLC ($r = 0.90$) results than with ELISA results ($r = 0.78$). The MTT bioassay, using a sensitive mammalian cell line, was demonstrated to be a useful method for quantification of deoxynivalenol as well as a potential toxicity screening method for contaminated extruded cereal-based products.

KEYWORDS: Deoxynivalenol; extrusion processing; detoxification; MTT bioassay; Chinese hamster ovary cell line; cytotoxicity

INTRODUCTION

Deoxynivalenol (vomitoxin), a type B trichothecene, occurs predominantly in grains such as wheat, barley, oats, rye, and maize and less often in rice, sorghum, and triticale. The occurrence of deoxynivalenol is associated primarily with *Fusarium graminearum* (telemorph *Gibberella zeae*) and *Fusarium culmorum*, both of which are important pathogens that cause *Fusarium* head blight in wheat and *Gibberella* ear rot in maize (*1*). Deoxynivalenol occurs throughout the world at unacceptably high concentrations in cereal grains in the United States, Canada, Europe, Asia, Africa, and New Zealand (*2*). Deoxynivalenol has also been detected in processed breakfast cereals with an average concentration of 100 ng/g (*3*) and in 50% of 92 snack food samples made from grain grown in the United States (*4*). Acute outbreaks of red mold toxicosis and gastroenteritis affecting humans and involving deoxynivalenol have been reported in China (*5*), India (*6*), and Japan (*7*). The main effect of deoxynivalenol at low dietary concentrations appears to be a reduction in food consumption (anorexia), whereas higher doses induce vomiting (emesis) (*8*). Deoxynivalenol can be both immunostimulatory and immunosuppressive in a variety of animal and cell culture models (*9*) and may be an etiological factor of glomerulonephritis/IgA nephropathy (*8*). Advisory levels for deoxynivalenol in finished food products for human consumption have been set at 1.0 μg/g in the United States and at 2.0 μg/g in uncleaned soft wheat in Canada (*10*). Estimated intake of deoxynivalenol from grains in the United States is a mean concentration of 25−53 μg/person/day (0.49−1.5 μg/kg of body weight/day) (*10*).

The removal of deoxynivalenol from foods and feeds remains an important objective worldwide due to their common occurrence and potential toxicity on humans and animals. The Food and Agriculture Organization (FAO) estimates that at least 25% of the world's cereal production is contaminated with mycotoxins (*11*). Economic losses from contaminated foods and feed are estimated to be annually in the billions of dollars worldwide (*11*). Detoxification strategies for contaminated foods and feeds to reduce or eliminate the toxic effects of deoxynivalenol by chemical, physical, and biological methods are crucial to improving food safety, preventing economic losses, and reclaiming contaminated products. Extrusion cooking of cereal products is being used increasingly as a method of processing cereals into breakfast foods, snack foods, and pet foods. Although deoxynivalenol is heat stable, the levels of deoxynivalenol in cereal-based foods were reduced between 27 and 95% by extrusion processing as determined by high-performance liquid chromatogarphy (HPLC) (*12*), gas chromatography (GC) (*13*), and enzyme-linked immunosorbent assay (ELISA) analysis (*14*). However, there remains a need to demonstrate that the toxicity or biological activity of deoxynivalenol has been

* Corresponding author [telephone (402) 472-2801; fax (402) 472-1693; e-mail lbullerman1@unl.edu].

10.1021/jf052443y CCC: $33.50
Published on Web 02/07/2006

reduced or completely eliminated in cereal-based foods using extrusion processing.

The comparison of in vivo and in vitro biological activities of deoxynivalenol demonstrated a significant correlation between these two tests (*15*). The cytotoxic effect of deoxynivalenol on Swiss mouse 3T3 fibroblasts and human diploid skin GM3349 fibroblast cell lines correlated well with weight loss and feed refusal observed in rat feeding studies (*15*). An in vitro tetrazoliaum salt (MTT) bioassay has already been demonstrated to be a useful method for screening contaminated grains to detect toxicity of *Fusarium* mycotoxins using sensitive mammalian cell lines (*16*−*18*). Deoxynivalenol was found to be a cytotoxic compound to a variety of cells including fibroblasts and lymphocytes (*17*, *18*). The Chinese hamster ovary (CHO-K1) cell line was found to be very sensitive to the cytotoxic effects of deoxynivalenol in the previous study (*18*). The MTT bioassay determines the ability of viable cells to convert the yellow tetrazolium salt to blue-colored formazan crystals by the mitochondrial dehydrogenase enzymes. It is rapid, versatile, quantitative, and highly reproducible mycotoxins screeing assay, lacking the need of any radioisotope and useful on a large scale-(*17*, *19*).

This study was done to determine whether deoxynivalenol concentrations in cereal-based foods were destroyed or converted to other derivatives that might remain biologically active or toxic during extrusion processing. The objective of this study was to determine loss of toxicity of deoxynivalenol in extruded cereal-based products by the MTT bioassay using a sensitive CHO-K1 cell line and to compare the results to chemical (HPLC) and biochemical (ELISA) methods of analysis.

MATERIALS AND METHODS

Production of Deoxynivalenol in Corn Grits. *F. graminearum* NRRL 5883 was obtained from the ARS Culture Collection National Center for Agricultural Utilization Research (USDA, Peoria, IL). The stock culture was maintained on carnation leaf agar (CLA) slants at 5 °C and was grown on CLA plates for 10 days at room temperature under daylight to enhance sporulation (*20*). Spores were harvested by adding sterile 0.05% Tween 80 to the culture plates and filtering through several layers of cheesecloth to remove mycelial debris and collect the spores. The number of spores in the spore suspension was determined by serial dilution and total plate count on potato dextrose agar. The spore suspension was then adjusted to contain $\sim 10^6$ spores/mL for inoculation.

Corn grits were obtained from Lauhoff Grain Co., Danville, IL. A total of 1200 g of corn grits was placed in jars and inoculated with 10^6 spores of *F. graminearum*/50 g of corn grits. The final moisture content of the corn grits was adjusted to 35% on a dry basis (db). The jars were shaken to provide homogeneous distribution of the spore suspension and then incubated at room temperature for deoxynivalenol production. After 15 days of incubation, the corn grits were autoclaved for 5 min to kill the spores and air-dried for 24 h. The corn grits were not exposed to longer autoclaving process to prevent the loss of toxin levels and any changes in the food matrix. All corn grits were pooled and mixed in a model C-100 low speed mixer (Hobart Corp., Troy, OH) to provide a homogeneous mixture of fermented grits and placed in an oven to dry at 65 °C overnight. The moisture content of the grits was adjusted to 20% (db) by adding distilled water and allowing the corn grits to equilibrate for 5 h before extrusion processing.

Extrusion Processing. A model CTFE-V laboratory scale conical twin-screw extruder (C. W. Brabender Instruments, Inc., Hackensack, NJ) with a barrel diameter of 1.9 cm and a length of 37 cm, a compression ratio of 3:1, corotating mixing screws, and a 3 mm diameter cylindrical die was used. A split−split plot design was used for the extrusion process at temperatures of 150, 175, and 200 °C and screw speeds of 70 and 140 rpm at 20% moisture content (db) to evaluate the reduction of deoxynivalenol concentrations in corn grits. All of the extruded samples were dried at 60 °C for 24 h, finely ground with a household blender, and kept frozen at −20 °C in airtight sample bags until analyzed.

The deoxynivalenol level in *F. graminearum* contaminated but unextruded corn grit extract was used as a positive control, whereas an extruded corn grit extract without any deoxynivalenol contamination was used as a negative control for HPLC and ELISA analysis. Deoxynivalenol (3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) standard was purchased from Sigma Chemical Co. (St. Louis, MO) and dissolved in HPLC-grade methanol. A recovery experiment was performed in triplicate by spiking blank extruded corn grits with the deoxynivalenol standard at a concentration of 15 μg/g. The spiked samples were left for 1 h before HPLC, ELISA, and MTT bioassay.

HPLC Analysis of Deoxynivalenol. Deoxynivalenol in contaminated extruded corn grits was analyzed as described by Cahill et al. (*21*) with minor modifications. DONtest-HPLC immunoaffinity columns (IAC) (Vicam, Watertown, MA) were used to clean up deoxynivalenol-contaminated corn samples because of the high specificity of the antibody-based columns. The contaminated extruded corn samples were extracted by adding 40 mL of deionized water and 2 g of polyethylene glycol 800 (PEG) to 10 g of the sample and blending at high speed for 3 min using a household blender. The extracts were filtered through both Whatman no. 1 filter papers and then Whatman 9 mm GF/A glass microfiber filters (VWR International Inc., Bristol, CT). The filtered extracts were diluted at a ratio of 1:1 with deionized water (1.0 mL = 0.125 g equivalent), and 1 mL of filtered extract of each sample was passed through the IAC at a rate of about 1 drop/s until air came through the column, using a Visiprep solid-phase extraction vacuum (Supelco, Bellefonte, PA). Subsequently, the columns were washed with 5 mL of deionized water, and the toxin was eluted by passing 1.0 mL of HPLC-grade methanol through the column. The eluates were collected in 2-dram amber vials. The eluates were evaporated to dryness under a gentle stream of nitrogen and redissolved in 1.5 mL of acetonitrile/water (10:90, v/v), and then 100 μL of the purified samples was injected into the HPLC system.

A model 510 HPLC (Waters Associates, Milford, MA) was equipped with a model 486 ultraviolet (UV) detector set at a wavelength 218 nm with 0.005 AUFS (Water Associates) and a model E 60 injector (Valco Instruments Co., Inc., Houston, TX) coupled with a model 728 automatic sampler (Micrometritics, Norcross, GA). A 150 × 3.9 mm i.d., 4 μm, reverse phase Nova-Pak C 18 column (Waters Associates) and a 10 × 3.9 mm i.d. Nova-Pak C 18 guard column (Waters Corp.) were used for chromatographic separations. The mobile phase consisting of a mixture of acetonitrile/water (10:90, v/v) was used at a flow rate of 1 mL/min. Chromatographic separations were monitored with computer-controlled Millennium 2010 software (Waters Associates) connected to the HPLC instruments. The detection limit for deoxynivalenol was 0.1 μg/g under the conditions tested. Concentrations of deoxynivalenol in the contaminated extruded corn samples were determined on the basis of retention times and peak areas compared to deoxynivalenol standards dissolved in the mobile phase.

The other possible *F. graminearum* products of deoxynivalenol derivatives, 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol, were not detected in the contaminated corn extracts after cleanup with DONtest-HPLC IAC when analyzed by HPLC as mentioned above. The same *F. graminearum* contaminated extruded corn extracts were analyzed after Zearala Test-HPLC IAC cleanup as described by Cetin et al. (*22*).

ELISA Analysis of Deoxynivalenol. Commercial Verotox quantitative ELISA test kits for deoxynivalenol (Neogen Corp., Lansing, MI) were used, following the standard protocols given for the kits with several modifications. Extruded corn samples were extracted by adding 50 mL of methanol/deionized water (50:50, v/v) to 10 g of ground sample and placing the mixture on a wrist-action shaker for 1 h. The methanol−water mixture was used for extraction instead of water alone due to the high water absorption capacity of extruded corn samples. The extracts were filtered through Whatman no. 1 filters, and 1 mL of the extracts was evaporated to dryness under a gentle stream of air and then redissolved in 1 mL of deionized water. The resulting solutions were diluted to a 1:10 ratio with deionized water, and 100 μL (1 mL

Table 1. Levels of Deoxynivalenol Remaining in *F. graminearum* Contaminated Corn Grits after Extrusion Processing As Measured by HPLC, ELISA, and MTT Bioassay

extrusion temperature and screw speed		remaining levels of deoxynivalenol in extruded corn grits, μg/g[a] (% reduction)[b]		
°C	rpm	HPLC	ELISA	MTT bioassay
150	70	18.42 ± 1.06^{cd} (21.7)	16.69 ± 1.02^{cde} (20.9)	17.55 ± 1.03^{cd} (21.1)
	140	17.43 ± 0.89^{cd} (25.8)	16.06 ± 0.95^{cde} (23.9)	17.23 ± 0.87^{cd} (24.1)
175	70	17.29 ± 0.71^{cd} (26.4)	15.57 ± 0.72^{cde} (26.2)	16.34 ± 1.04^{cd} (26.6)
	140	16.93 ± 1.07^{cd} (27.9)	15.26 ± 0.86^{cde} (27.7)	16.10 ± 0.77^{cd} (27.6)
200	70	16.20 ± 0.77^{de} (31.1)	14.96 ± 1.01^{def} (29.0)	14.85 ± 1.02^{de} (33.3)
	140	15.26 ± 0.68^{ef} (35.0)	13.90 ± 0.63^{efg} (34.1)	14.12 ± 0.72^{ef} (36.6)

[a] Means ± standard deviations ($n = 3$). Means with different letters in the same columns were significantly different as calculated from the least significant mean (LSM) comparison at 95% confidence interval ($P < 0.05$). [b] Means of three replications ($n = 3$) calculated from the initial levels of deoxynivalenol.

= 0.018 g equivalent) of each sample was analyzed by the ELISA test kits with a limit of detection of 0.1 μg/g.

Cell Culture and Conditions. The CHO-K1 was obtained from Dr. T. Lawson (Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE). Dulbecco's modified Eagle medium (DMEM), with nutrient mixture F-12 (Ham) (1:1), fetal bovine serum (FBS) heat inactivated, phosphate-buffered saline (PBS), pH 7.2, 1 M HEPES buffer solution, 100 mM sodium pyruvate solution, sodium bicarbonate 7.5% (w/v), 10 mM nonessential amino acid (NEAA), trypsin-EDTA (0.25% trypsin, 1 mM EDTA·4Na), and antibiotic−antimycotic (10000 units of penicillin, 10000 μg of streptomycin, and 25 μg of amphotericin B as fungizone, antimycotic in 0.85% saline) were obtained from Life Technologies Gibco BRL Products (Rockville, MD). CHO-K1 cells were grown as a monolayer in DMEM/F-12 in 80 cm^2 culture flasks and used at passage numbers between 40 and 50. DMEM/F-12 was supplemented with 1.5 g/L sodium bicarbonate, 0.11 g/L sodium pyruvate, 1% NEAA, 25 mM HEPES, antibiotic−antimycotic (100 units of penicillin/mL, 100 μg of streptomycin/ml, and 250 ng of amphotericin B/mL), and 10% FBS. The CHO-K1 monolayers were harvested when they reached 80% confluence to maintain exponential growth.

MTT Bioassay. The MTT bioassay determines the ability of viable cells to reduce the yellow tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) to blue formazan crystals by mitochondrial enzymes. The concentration of formazan crystals can be spectrophotometrically determined when dissolved in an organic solvent (*23*). Deoxynivalenol standards and *F. graminearum* contaminated extruded corn extracts cleaned up with IAC were transferred to 2-dram amber vials and evaporated to dryness at 50 °C under a stream of nitrogen and then resuspended in DMEM/F-12 containing 0.1% ethanol. The samples were filtered through Acrodisc 0.22 μm syringe filters (Gelman Sciences, Ann Arbor, MI) for sterilization. The CHO-K1 cell monolayer in exponential growth was harvested using 0.25% trypsin, and a single-cell suspension was obtained by repeated pipetting. The cells were counted with a hemacytometer (Hausser Scientific, Horsham, PA) under the microscope, and the MTT bioassay was performed concominantly. The MTT bioassay measures the viable cells in target cells during the exponential growth phase. To determine the seeding cell number of CHO-K1 in the exponential phase of growth throughout the experiment, the single-cell suspensions containing cell densities ranging from 1×10^2 to 1×10^5 cells in 200 μL of DMEM/F-12 medium/well were added to 96-well plates by serial dilution. The plates were incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. MTT, sodium dodecyl sulfate (SDS), and *N*,*N*-dimethylformamide (DMF) were purchased from Sigma Chemical Co. (St. Louis, MO). MTT was dissolved in PBS at a concentration of 5 mg/mL, filtered through a 0.22 μm filter to sterilize and remove insoluble residues, and then stored in the amber vials at 4 °C for up to 1 month. After 72 h of incubation, 25 μL of the MTT solution was added to each well of the 96-well plates and incubated for 4 h at 37 °C in a humidified atmosphere of 5% CO_2. At the end of the incubation period, the media were discarded using a suction pump. The extraction buffer of 20% w/v SDS in a solution of 50% DMF in demineralized water (50:50, v/v) was prepared and adjusted to pH 4.7 by adding 80% acetic acid and 1 N HCl. It was filtered through a 0.22 μm filter to sterilize and remove insoluble residues and stored at room temperature. The extraction buffer of 100 μL of 20% SDS was added into each well of the 96-well plate to solubilize the formazan crystals (*23*). The 96-well plates were placed on an orbital shaker at 37 °C overnight. The absorbance was measured at a test wavelength of 570 nm using a Sunrise absorbance microplate reader (Tecan, Phoenix, CA). The number of seeding cells was found to be 5×10^3 cells/well from the linear correlation between the number of seeded cells and the optical density (OD) values.

The cytotoxic effects of deoxynivalenol were measured by performing the MTT bioassay. The CHO-K1 monolayer was trypsinized and plated into 96-well plates at a cell density of 5×10^3 cells/well in 100 μL of DMEM/F-12 medium/well. They cells were allowed to attach to the wells for 24 h of incubation at 37 °C in a humidified atmosphere of 5% CO_2. The deoxynivalenol standards ranged from 0.04 to 20 μg/mL, or the extracts of extruded corn samples (1 mL = 0.125 g equivalent) in 100 μL of DMEM/F-12 medium containing 0.1% ethanol were added to the 96-well plates and incubated for 48 h at 37 °C in a humidified atmosphere of 5% CO_2. After 48 h of incubation, 25 μL of the MTT solution was added to each well of the 96-well plates and incubated for 4 h at 37 °C in a humidified atmosphere of 5% CO_2, and then the media were discarded using a suction pump. The extraction buffer of 100 μL of 20% SDS was added into each well, and the 96-well plates were placed on an orbital shaker at 37 °C overnight. The absorbance was measured at a test wavelength of 570 nm using a microplate reader. The positive control contained contaminated unextruded corn extracts and the CHO-K1 cells in the medium containing 0.1% ethanol. The negative control contained the CHO-K1 cells and uncontaminated extruded corn extracts in the culture medium containing 0.1% ethanol.

The reduced levels of deoxynivalenol in extruded corn samples were calculated from the linear regression equation ($y = ax + b$) from the dose−response curve plotted using the concentrations of deoxynivalenol standard in micrograms per milliliter versus OD values. The inhibition concentration of 50% cell proliferation (IC_{50}) of deoxynivalenol was calculated by locating the *x*-axis value corresponding to half the absorbance value of the control (*19*).

Statistical Analysis. All trials were repeated three times. The Statistical Analysis System (SAS) was used to verify significant differences between the treatments by analysis of variance in the mixed procedure, followed by the comparison of the least-squares means (LSM) at 95% confidence intervals for the extrusion study.

RESULTS

The effects of extrusion temperatures and screw speeds on percent reduction of deoxynivalenol levels in *F. graminearum* contaminated corn grits were found to be significant when calculated from their differences of LSM values among HPLC, ELISA, and the MTT bioassay ($P < 0.05$) (**Table 1**). The results of percent remaining deoxynivalenol in contaminated corn grits after extrusion processing at temperatures of 150, 175, and 200 °C and screw speeds of 70 and 140 rpm measured by HPLC, ELISA, and MTT bioassay are presented in **Figure 1**. The effect

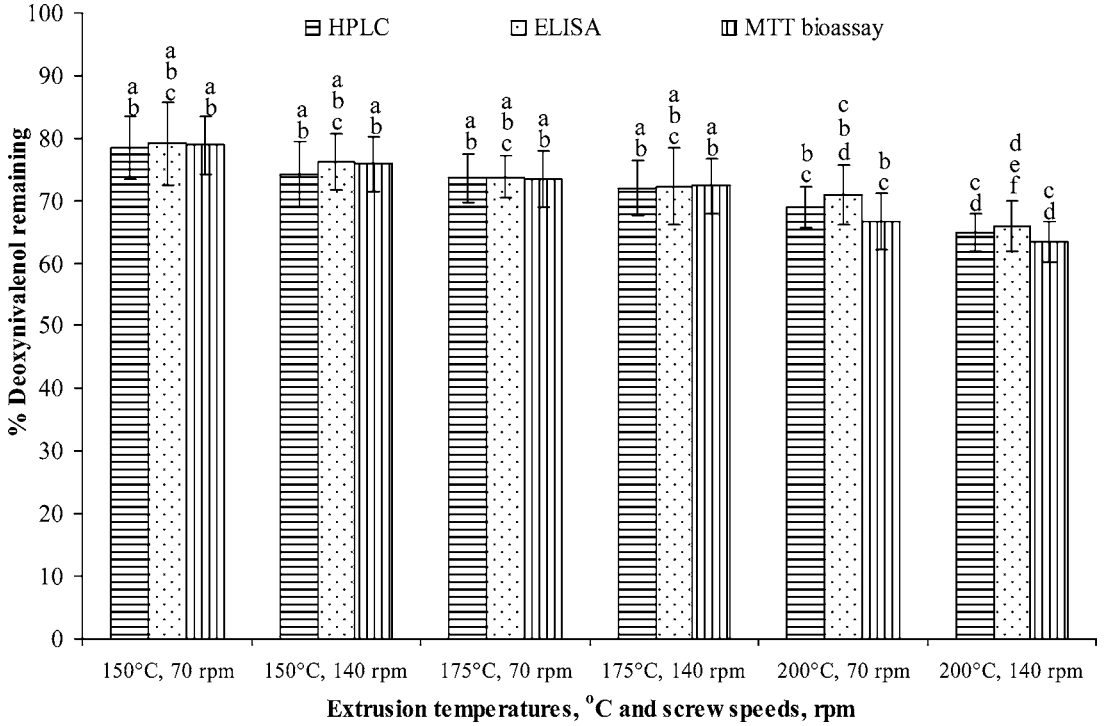

Figure 1. Results of percent remaining levels in contaminated corn grits after extrusion processing at temperatures of 150, 175, and 200 °C and screw speeds of 70 and 140 rpm measured by HPLC, ELISA, and MTT bioassay. Each bar represents the mean of percent deoxynivalenol remaining ± SD values of extruded samples ($n = 3$), and bars with different letters within each method of analyses are significantly different ($P < 0.05$). The mean values of percent deoxynivalenol remaining in *F. graminearum* contaminated corn (positive control) and extruded blank corn extracts (negative control) were calculated as 100 and 0%, respectively.

of extrusion temperatures, which showed a linear response, was found to be more effective on detoxification of deoxynivalenol than the extrusion screw speeds. The initial levels of deoxynivalenol in *F. graminearum* contaminated corn grits used as a positive control were found at mean concentrations and standard deviations (SD) of 23.50 ± 2.47, 21.11 ± 3.28, and 22.26 ± 2.63 μg/g ($n = 3$) as measured by HPLC, ELISA, and MTT bioassay, respectively. The deoxynivalenol levels in negative control, extruded blank corn grits without any *F. graminearum* contamination, were found to be zero as measured by all three methods of analysis. The other possible *F. graminearum* products of deoxynivalenol derivatives and zearalenone were not detected in the contaminated corn extracts after cleanup with DONtest-HPLC IAC when analyzed by HPLC (*21*, *24*). The same *F. graminearum* contaminated extruded corn extracts were analyzed after Zearala Test-HPLC IAC cleanup, and zearalenone was found at a mean concentration of 37.88 μg/g as measured by HPLC (*22*). The percent recoveries of HPLC, ELISA, and the MTT bioassay were found at mean values and SD of 90 ± 3.74, 98 ± 3.81, and 101 ± 5.52%, respectively. The limits of detection for HPLC, ELISA, and MTT bioassay were found to be 0.1, 0.1, and 0.07 μg/g, respectively. The average of repeatabilities, relative standard deviations (RSDr), of HPLC, ELISA, and the MTT bioassay by analyzing triplicate samples ($n = 3$) were found to be 5.8% (ranging from 4.2 to 7.4%), 4.9% (ranging from 3.3 to 6.5%), and 6.2% (ranging from 5.8 to 7.6%), respectively.

The reduction of deoxynivalenol concentrations by extrusion processing at temperatures of 150, 175, and 200 °C was by 24, 27, and 33%, respectively, as analyzed by HPLC (**Table 1**). The maximum reduction of deoxynivalenol of 35% at 200 °C and 140 rpm was found to be significantly higher than the minimum reduction level of 22% at 150 °C and 70 rpm in contaminated corn grits when measured by HPLC ($P < 0.05$). The extrusion temperature of 200 °C reduced deoxynivalenol levels more effectively than did temperature of 150 and 175 °C. The extrusion screw speed of 140 rpm was found to be not significantly more effective on reduction of deoxynivalenol levels in contaminated corn grits than the screw speed of 70 rpm ($P > 0.05$).

Deoxynivalenol concentrations by extrusion processing at temperatures of 150, 175, and 200 °C were reduced by 22, 27, and 32%, respectively, as analyzed by ELISA (**Table 1**). The maximum reduction of deoxynivalenol of 34% at 200 °C and 140 rpm was found to be significantly higher than the minimum reduction level of deoxynivalenol of 21% at 150 °C and 70 rpm as measured by ELISA ($P < 0.05$). The extrusion temperature of 200 °C reduced deoxynivalenol levels in corn grits more effectively than did temperatures of 150 and 175 °C. The effects of extrusion screw speeds of 140 and 70 rpm on reduction of deoxynivalenol levels in contaminated corn grits were not found to be significantly different ($P > 0.05$).

The quantification of remaining deoxynivalenol levels in contaminated corn samples was also analyzed by the MTT bioassay using the CHO-K1 cell line. The cytotoxic effects of deoxynivalenol standards on the CHO-K1 cell line at concentrations of 0.04−20 μg/mL are shown in **Figure 2** after 48 h of exposure. The IC_{50} value of deoxynivalenol was found at a concentration of 0.27 μg/g after 48 h of exposure. The reduction of deoxynivalenol concentrations in contaminated extruded corn samples was calculated using a linear regression equation from the dose−response curve of deoxynivalenol (**Figure 2**). The OD values of contaminated unextruded corn extracts (positive control), extruded blank corn extracts (negative control), and contaminated extruded corn extracts are given in **Figure 2**. The deoxynivalenol concentrations at extrusion temperatures of 150, 175, and 200 °C were reduced by 23, 27, and 35%, respectively (**Table 1**). The maximum reduction of deoxynivalenol of 37%

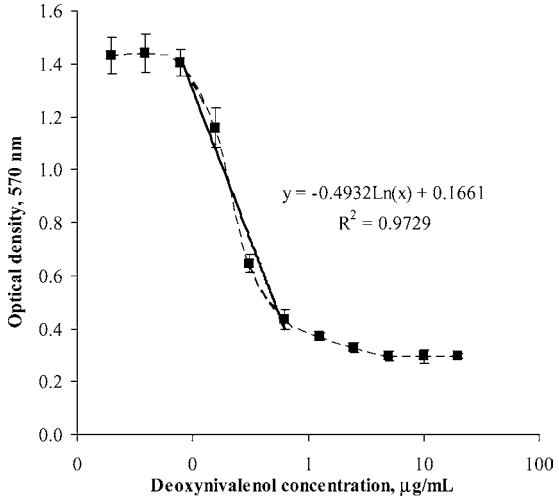

Figure 2. Dose–response curve of deoxynivalenol at concentrations of 0.04–20 μg/mL determined by MTT bioassay using the CHO-K1 cell line after 48 h of exposure. The mean OD ± SD values of the extruded samples ($n = 3$) at 150, 175, and 200 °C and screw speeds of 70 and 140 rpm were found to be 0.866 ± 0.030, 0.885 ± 0.028, 901 ± 0.031, 0.908 ± 0.029, 0.949 ± 0.034, and 0.973 ± 0.025, respectively, as measured by MTT bioassay. The OD values of *F. graminearum* contaminated corn (positive control) and extruded blank corn extracts (negative control) were found to be 0.751 ± 0.048 and 1.282 ± 0.050, respectively.

at 200 °C and 140 rpm was found to be significantly higher than the minimum reduction level of deoxynivalenol of 21% at 150 °C and 70 rpm ($P < 0.05$). The extrusion screw speed of 140 rpm was found to be not significantly more effective on the reduction of deoxynivalenol levels in contaminated corn grits than the screw speed of 70 rpm ($P > 0.05$). MTT bioassay results were more closely correlated with HPLC ($r = 0.90$) results than with ELISA ($r = 0.78$) results. The MTT bioassay was found to be useful as a quantitative method for deoxynivalenol with the detection limit of 0.07 μg/g, as well as for screening purposes to evaluate the reduced toxicity of deoxynivalenol to a mammalian cell culture. The effects of extrusion processing on deoxynivalenol stability in artificially contaminated corn grits fermented with *F. graminearum* were measured by HPLC and ELISA, and the loss of toxicity of deoxynivalenol was confirmed by the MTT bioassay using the deoxynivalenol sensitive CHO-K1 cell line.

DISCUSSION

A number of physical, chemical, and biological detoxification methods have been attempted to reduce deoxynivalenol levels in naturally or artificially contaminated grains. The use of chemicals is one strategy to detoxify deoxynivalenol in contaminated grains; however, human and animal health concerns and ecological and economic problems are quite high, in addition to altering the food physically, organoleptically, or nutritionally (*25*, *26*). Since the scab outbreak in Ontario wheat in 1980, a number of physical and chemical procedures have been attempted to reduce deoxynivalenol in *Fusarium*-contaminated grains, including cleaning steps prior to milling, removal of infected kernels, and various forms of heat treatment (*27*, *28*). Physical treatments for removing deoxynivalenol in grains by thermal processing such as baking, roasting, microwave heating, spray drying, cooking, frying, and extrusion processing have been found to have varying degrees of success. Deoxynivalenol has been found in a variety of processed food products such as bread, corn flakes, coffee, and beer due to heat stability (*29*−*31*); therefore, it is critical to optimize the food-processing conditions needed to detoxify contaminated grains to ensure adequate food safety and health. In previous studies of heat treatments, deoxynivalenol was not destroyed during the baking of different ethnic products such as Egyptian bread, Western-style bread, or cookies baked from hard wheat flour (*32*). El-Banna et al. (*33*) also reported that baking flour into bread failed to destroy deoxynivalenol. However, in other studies deoxynivalenol levels were reported to be reduced up to 35% (*34*) and 44% (*30*) during the baking process. Deoxynivalenol levels were also reduced up to 50−60% in cooked pasta and noodles because of leaching into the cooking water (*35*). The percent reductions of deoxynivalenol and 15-acetyldeoxynivalenol during the preparation of tortillas with 2% lime water used to boil corn were found to be 72−82 and 100%, respectively (*36*). Deoxynivalenol was reduced in the range of 60−100% by treatment with aqueous bicarbonate solution at 10, 20, or 50% of the ground maize and subsequent heating at 80 or 110 °C for 2 and 12 days (*37*). Wolf-Hall and Bullerman (*38*) reported that deoxynivalenol was found to be heat stable at temperatures of 100 or 120 °C and at acidic pH 4.0, whereas it was completely destroyed at temperatures of 120 and 170 °C and at alkaline pH 10.0 after 30 and 15 min, respectively.

One of the more promising physical methods is extrusion processing under high temperature, high pressure, and high shear stress to detoxify deoxynivalenol in cereal-based food products. The degree of detoxification of deoxynivalenol depends on the combination of temperatures and screw speeds, the moisture content, the initial mycotoxin concentration of materials, the chemical stability of mycotoxin, and its binding ability to the food matrices during extrusion processing. It has been shown to be quite effective in reducing 95% of fumonisin B_1 (*39*), 53% of aflatoxin (*40*), 95% of deoxynivalenol (*12*), and 83% of zearalenone (*22*) in corn-based foods. The application of extrusion processing might be very effective in detoxifying multiple *Fusarium* toxins due to their widespread natural co-occurrence in cereals.

Several studies were done to evaluate the effects of extrusion processing on the reduction of deoxynivalenol. Wolf-Hall et al. (*14*) studied the effects of extrusion processing on 4 and 5.47 μg/g deoxynivalenol-spiked corn grits and dog food at a moisture content of 22% and a temperature of 100 °C and screw speeds of 140 and 29 rpm, respectively. There were no significant reductions in deoxynivalenol concentrations observed. However, the canning of spiked cream-style corn resulted in a 12% loss of deoxynivalenol by autoclaving at 121 °C for 87 min (*14*). The extrusion temperature was much lower than those used this study; therefore, it might be the main reason no loss of deoxynivalenol was observed. Another study of the effects of extrusion cooking coupled with sodium bisulfite in naturally contaminated wheat containing 7.3 μg/g of deoxynivalenol showed that soaking of contaminated wheat in water and 5% sodium bisulfite treatments resulted in 33−96% reductions in deoxynivalenol levels. On the other hand, extrusion processing at temperatures up to 170 °C and 16.5% moisture content reduced deoxynivalenol by 11% from the starting level (*13*). The reason for lower reduction of deoxynivalenol than in the present study might be due to the lower extrusion temperatures and screw speeds and the moisture content applied. In addition, it might be attributed to the different type of deoxynivalenol contamination (naturally versus artificially contaminated with *F. graminearum*), differences between the food matrices (wheat versus corn grits), and the starting levels of deoxynivalenol concentrations in wheat and corn grits at

concentrations of 7.3 and 23.5 μg/g, respectively. Cazzaniga et al. (*12*) also studied the effect of extrusion cooking on detoxification of corn flour spiked with 5 ppm of deoxynivalenol containing 15 and 30% moisture contents, temperatures of 150 and 180 °C, and a screw speed of 120 rpm. Extrusion processing was found to be effective for detoxification of deoxynivalenol by 95% reduction under the extrusion conditions studied. The results of these studies showed greater reduction of deoxynivalenol levels after extrusion processing than obtained during the present study, possibly due to the same factors as mentioned above.

In this study the effects of extrusion temperatures and screw speeds caused significant reduction in deoxynivalenol levels ranging from 21 to 35% in corn grits containing 20% moisture. The effect of extrusion processing at a temperature of 200 °C was found to be greater than that at temperatures of 150 and 175 °C on reductions of deoxynivalenol levels in artificially contaminated corn grits. Extrusion temperature effects on reduction of deoxynivalenol levels were found to occur in a linear fashion. Extrusion screw speeds of 70 and 140 rpm showed no significantly different effects on reduction of deoxynivalenol levels in contaminated corn grits. Although deoxynivalenol derivatives were also analyzed by HPLC to evaluate their possible cross-reactivity in biological analysis, they were not found in the corn grit extracts. *F. graminearum* also produced zearalenone in the corn grits, but it was analyzed separately as another study (*22*). There is still a need to determine more precisely the optimum extrusion processing conditions coupled with addition of chemicals such as vitamins and food additives to improve food quality and safety and to eliminate or remove deoxynivalenol in a practical approach. The application of extrusion processing under optimized conditions might prevent considerable economic losses in contaminated agricultural products by detoxifying deoxynivalenol-contaminated cereal grains. This research may provide crucial information for risk assessment of extruded cereal-based foods, such as snack and breakfast foods, which are commonly consumed by children. Residual levels of deoxynivalenol in extruded products may cause long-term chronic toxicity due to accumulation in target organs. Therefore, the use of suitable in vitro bioassays using sensitive mammalian cell lines may provide essential toxicity data about deoxynivalenol that would show that chemical changes occurring during extrusion processing can be correlated to biological transformations and loss of toxicity.

The MTT bioassay was applied as a quantitative method and also showed the reduced toxicity of deoxynivalenol to mammalian cell cultures after extrusion processing. The CHO-K1 cell line was shown to be a sensitive model to detect the cytotoxic effects of deoxynivalenol. The MTT bioassay results were more closely correlated with the HPLC results than with the ELISA results. The more rapid MTT bioassay is unlike many of the chemical analyses in that it does not require high capital costs for equipment (e.g., gas chromatography, HPLC, and mass spectroscopy) or a highly skilled technician. This study showed that the MTT bioassay using a sensitive mammalian cell line is a useful method for the quantification of deoxynivalenol as well as a potential cytotoxicity screening method for deoxynivalenol-contaminated extruded cereal-based products to prove reductions of toxicity and biological activity.

ABBREVIATIONS USED

CHO-K1, Chinese hamster ovary cell line; ELISA, enzyme-linked immunosorbant assay; IAC, immunoaffinity column; MTT, tetrazolium salt.

ACKNOWLEDGMENT

We thank Dr. Kent Eskeridge for assistance during the statistical analysis. We are grateful to Dr. Milford A. Hanna and his laboratory staff for assistance and Dr. Dojin Ryu for helpful suggestions during extrusion processing.

LITERATURE CITED

(1) D'Mello, J. P. F.; Placinta, C. M.; Macdonald, A. M. C. *Fusarium* mycotoxins: a review of global implications for animal health, welfare and productivity. *Anim. Feed Sci. Technol*. **1999**, *80*, 183−205.

(2) Placinta, C. M.; D'Mello, J. P. F.; Macdonald, A. M. C. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Anim. Feed Sci. Technol*. **1999**, *78*, 21−37.

(3) Trucksess, M. W.; Flood, M. T.; Page, S. W. Thin-layer chromatography determination of deoxynivalenol in processed grain products. *J. Assoc. Off. Anal. Chem*. **1986**, *69*, 35−36.

(4) Brumley, W. C.; Trucksess, M. W.; Adler, S. H.; Cohen, C. K.; White, K. D.; Sphon, J. A. Negative ion chemical ionization mass spectrometry of deoxynivalenol (DON): application to identification of DON in grains and snack foods after quantitation/isolation by thin-layer chromatography. *J. Assoc. Off. Anal. Chem.* **1985**, *33*, 326−330.

(5) Huang, S. X. Mycotoxicoses occurring in flooded areas and preventive measures against them. In *Proceedings on Chinese Counter measures for Anti-epidemic and Disaster Relief*; Chinese Ministry of Health: Beijing, China, 1992; pp 45−49.

(6) Bhat, R. M.; Beedu, S. P.; Ramakrishna, Y.; Munshi, K. L. Outbreak of trichothecene mycotoxicosis associated with consumption of mold-damaged wheat products in Kashmir Valley, India. *Lancet* **1989**, *7*, 35−37.

(7) Yoshizawa, T.; Takeda, H.; Ohi, T. Structure of a novel metabolite from deoxynivalenol, a trichothecene mycotoxin, in animals. *Agric. Biol. Chem*. **1983**, *47*, 2133−2135.

(8) Rotter, B. A.; Prelusky, D. B.; Pestka, J. J. Toxicology of deoxynivalenol (vomitoxin). *J. Toxicol. Environ. Health* **1996**, *48*, 1−34.

(9) Pestka, J. J.; Bondy, G. S. Mycotoxin-induced immune modulation. In *Immunotoxicology and Immunopharmacology*, 2nd ed.; Dean, J. H., Luster, M. I., Munson, A. E. E., Kimber, I., Eds.; Raven Press: New York, 1994; pp 163−183.

(10) JECFA. *53rd Report. Safety Evaluation of Certain Food Additives and Contaminants*; WHO Food Additives 47; Joint FAO/WHO Expert Committee on Food Additives; FAO: Rome, Italy, 2001.

(11) Pohland, A. E. Mycotoxins in review. *Food Addit. Contam.* **1993**, *10*, 17−28.

(12) Cazzaniga, D.; Basilico, J. C.; Gonzalez, R. J.; Torres, R. L.; de Greef, D. M. Mycotoxins inactivation by extrusion cooking of corn flour. *Lett. Appl. Microbiol*. **2001**, *33*, 144−147.

(13) Accerbi, M.; Rinaldi, V. E. A.; Ng, P. K. W. Utilization of highly deoxynivalenol-contaminated wheat via extrusion processing. *J. Food Prot*. **1999**, *62*, 1485−1487.

(14) Wolf-Hall, C. E.; Hanna, M. A.; Bullerman, L. B. Stability of deoxynivalenol in heat treated products. *J. Food Prot.* **1999**, *62*, 962−964.

(15) Abbas, H. K.; Mirocha, C. J.; Shier, T. Mycotoxins produced from fungi isolated from foodstuffs and soil: comparison of toxicity in fibroblasts and rat feeding tests. *Appl. Environ. Microbiol.* **1984**, *48*, 654−661.

(16) Visconti, A.; Minervini, F.; Lucivero, G.; Gambatesa, V. Cytotoxic and immunotoxic effects of *Fusarium* mycotoxins using a rapid colorimetric bioassay. *Mycopathologia* **1991**, *113*, 181−186.

(17) Hanelt, M.; Gareis, M.; Kollarczik, B. Cytotoxicity of mycotoxins evaluated by the MTT-cell culture assay. *Mycopathologia* **1994**, *128*, 167−174.

(18) Cetin, Y.; Bullerman, L. B. Cytotoxicity of *Fusarium* mycotoxins to mammalian cell cultures as determined by the MTT bioassay. *Food Chem. Toxicol.* **2005**, *43*, 755−764.
(19) Dombrink-Kurtzman, M. A.; Bennet, G. A.; Richard, J. L. An optimized MTT bioassay for determination of cytotoxicity of fumonisins turkey lymphocytes. *J. AOAC Int.* **1994**, *77*, 512−516.
(20) Nelson, P. E.; Toussoun, T. A.; Marasas, W. F. O. *Fusarium Species: An lllustrated Manual for Identification*; The Pennsylvania State University Press: University Park, PA, 1983.
(21) Cahill, L. M.; Kruger, S. C.; McAlice, B. T.; Ramsey, C. S.; Prioli, R.; Kohn, B. Quantification of deoxynivalenol in wheat using an immunoaffinity column and liquid chromatography. *J. Chromatogr. A* **1999**, *859*, 23−28.
(22) Cetin Y.; Bullerman, L. B. Evaluation of reduced toxicity of zearalenone by extrusion processing as measured by the MTT cell proliferation assay. *J. Agric. Food Chem.* **2005**, *53*, 6558−6563.
(23) Hansen, M. B.; Nielsen, S. E.; Berg, K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J. Immunol. Methods* **1989**, *119*, 203−210.
(24) Visconti, A.; Pascale, M. Determination of zearalenone in corn by means of immunoaffinity clean-up and high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. A* **1998**, *815*, 133−140.
(25) Beaver, R. W. Decontamination of mycotoxin-containing foods and feedstuffs. *Trends Food Sci. Technol*. **1991**, 170−173.
(26) Jackson, L. S.; Bullerman, L. B. Effect of processing on *Fusarium* mycotoxins. *Impact of Processing on Food Safety*; Kluwer Academic/Plenum Publishers: New York, 1999; pp 243−261.
(27) Bennett, G. A.; Richard, J. L. Influence of processing on *Fusarium* mycotoxins in contaminated grains. *Food Technol.* **1996**, 235−238.
(28) Charmley, L. L.; Prelusky, D. B. Decontamination of *Fusarium* mycotoxins. In *Mycotoxins in Grains. Components Other than Aflatoxins*; Miller, J. D., Trenholm, H. L., Eds.; Eagan Press: St. Paul, MN, 1994; pp 421−433.
(29) Scott, P. M.; Kanhere, R. S.; Weber, D. Analysis of Canadian and imported beers for *Fusarium* mycotoxins by gas chromatography−mass spectrometry. *Food Addit. Contam.* **1993**, *10*, 381−389.
(30) Neira, M. S.; Pacin, A. M.; Martinez, E. J.; Molto, G.; Resnik, S. L. The effects of bakery processing on natural deoxynivalenol contamination. *Int. J. Food Microbiol*. **1997**, *37*, 21−25.
(31) Molto, G.; Samar, M. M.; Resnik, S.; Martinez, E. J.; Pacin, A. Occurrence of trichothecenes in Argentinean beer. A preliminary exposure assessment. *Food Addit. Contam*. **2000**, *17*, 809−813.
(32) Scott, P. M. Effects of food processing on mycotoxins. *J. Food Prot*. **1984**, *47*, 489−499.
(33) El-Banna, A. A.; Lau, P. Y.; Scott, P. M. Fate of mycotoxins during processing of food stuffs. II. Deoxynivalenol (vomitoxin) during making of Egyptian bread. *J. Food Prot*. **1983**, *46*, 484−484.
(34) Young, J. C.; Fulcher, R. G.; Hayhoe, J. H.; Scott, P. M.; Dexter, J. E. Effect of milling and baking on deoxynivalenol (vomitoxin) content of eastern Canadian wheats. *J. Agric. Food Chem.* **1984**, *32*, 659−664.
(35) Nowicki, T. W.; Gaba, D. G.; Dexter, J. E.; Matsuo, R. R.; Clear, R. M. Retention of *Fusarium* mycotoxin deoxynivalenol in wheat during processing and cooking of spaghetti and noodles. *J. Cereal Sci*. **1988**, *8*, 189−202.
(36) Abbas, H. K.; Mirocha, C. J.; Rosiles, R.; Carvajal, M. Decomposition of zearalenone and deoxynivalenol in the process of making tortillas from corn. *Cereal Chem*. **1988**, *65*, 15−19.
(37) Lauren, D. R.; Smith, W. A. Stability of the fusarium mycotoxins nivalenol, deoxynivalenol and zearalenone in ground maize under typical cooking environments. *Food Addit. Contam*. **2001**, *18*, 1011−1016.
(38) Wolf-Hall, C. E.; Bullerman, L. B. Heat and pH alter the concentration of deoxynivalenol in an aqueous environment. *J. Food Prot*. **1998**, *61*, 365−367.
(39) Katta, S. K.; Jackson, L. S.; Sumner, S. S.; Hanna, M. A.; Bullerman, L. B. Effect of temperature and screw speed on stability of fumonisin B_1 in extrusion-cooked corn grits. *Cereal Chem*. **1999**, *76*, 16−20.
(40) Elias-Orozco, R.; Castellanos-Nava, A.; Gaytán-Martínez, M.; Figueroa-Cárdenas, J. D.; Loarca-Piña, G. Comparison of nixtamalization and extrusion processes for a reduction in aflatoxin content. *Food Addit. Contam.* **2002**, *19*, 878−885.

Received for review October 3, 2005. Revised manuscript received January 8, 2006. Accepted January 10, 2006. This research was conducted under Project 16097 of the Agricultural Research Division, University of Nebraska−Lincoln, Lincoln, NE, and is based upon work supported in part by grants from the Midwest Advanced Food Manufacturing Alliance and the Anderson Fund of NC 213. Journal Series 15082.

JF052443Y