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Heat Stability of Zearalenone in an Aqueous Buffered Model System

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Zearalenone is an endocrine disruptor with estrogenic activity, produced primarily by *Fusarium graminearum*, a common cause of corn ear rot and *Fusarium* head blight or scab in wheat. Zearalenone can be a contaminant of both corn and wheat and may survive thermal food processes. This study was done to determine the heat stability of zearalenone. Reduction of zearalenone was measured during heating at different temperatures (100, 125, 150, 175, 200, and 225 °C) in an aqueous buffer solution at different pH values. The rate and extent of zearalenone reduction increased with processing temperature. Less than 23% of zearalenone was lost when heated to ≤125 °C whereas 34−68% was lost at 150 °C after 60 min, depending on the pH of the buffer. Greater than 92% of zearalenone was lost after 60 min when heated to ≥175 °C, and complete reduction of zearalenone was observed in less than 30 min at 225 °C, regardless of pH. Overall, zearalenone was most stable at pH 7 followed by that at pH 4 and 10, and the greatest losses occurred above 175 °C.

KEYWORDS: Zearalenone; reduction; heat processing; pH; kinetics

INTRODUCTION

Zearalenone is a unique mycotoxin with estrogenic activity that can disrupt the function of the endocrine hormone estrogen in animals and possibly humans. This mycotoxin, frequently found in all major cereal grains worldwide, is a result of infection and growth of ubiquitous fungi such as *Fusarium graminearum* and *F. culmorum* (*1*). The toxicity of zearalenone is due to its estrogenic activity that can manifest itself as hyperestrogenism in swine with symptoms of infertility, stillbirths, abortions, and rectal or vaginal prolapses (*2*). The significance of endocrine modulation is mainly attributed to the fact that altered hormonal balance may affect the carcinogenic process in endocrine sensitive tissues including adrenals, thyroid, prostate, and breast (*3*).

Zearalenone, 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcyclic acid lactone (*4*), is also known to be a very heat-stable compound despite its large lactone ring (**Figure 1**). Efforts have been made to reduce the level of zearalenone by various chemical, physical, and biological processing methods. In common heat processing, neither pure zearalenone nor zearalenone present in ground corn was decomposed at 150 °C for 44 h (*5*). In another study, zearalenone decomposed only by 3.2% after heating for 15 min at 100 °C and 28.5% after 60 min at 150 °C, when added to wheat flour containing water (35%) at a level of 20 μg/g, (*6*). They also reported reductions of zearalenone of 37% and 69% after 30 and 60 min, respectively, when artificially contaminate zearalenone in wheat flour was heated at 200 °C. In another processing study using an extruder, the reduction of zearalenone in corn grits ranged from 66% to 83% at temperatures of 120−160 °C (*7*).

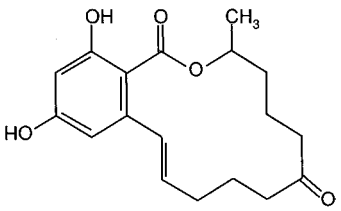

Figure 1. Structure of zearalenone.

Since zearalenone may be found in cereal grains destined for both human and animal consumption, information about its stability in thermal processes and efficient decontamination procedures are needed. The objective of this study was to determine the thermal stability of zearalenone in an aqueous environment at acidic, neutral, and basic pH levels, thus eliminating the possibility of interaction with a cereal grain matrix.

MATERIALS AND METHODS

Materials. Teorell and Stenhagen's citrate−phosphate−borate buffer (*8*) was prepared and adjusted to the three pH levels of 4.0, 7.0, and 10.0. One batch of buffer solution was made for each pH level and

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replication. Stock solution of zearalenone was prepared by dissolving purified zearalenone (Sigma Chemical Co., St. Louis, MO) in methanol at 5 mg/mL and then adding the mixture to buffer solution to achieve a final concentration of 2 μg/mL. All reagents were of analytical grade, and solvents were of HPLC grade.

Processing of the Model System. A volume of 250 mL of the Teorell and Stenhagen's citrate−phosphate−borate buffer solution containing zearalenone was placed in a 450 mL stainless steel pressurized Parr heat reactor vessel (Parr Instrument Company, Moline, IL) and heated to processing temperatures of 100, 125, 150, 175, 200, and 225 °C with an electric heating mantle. Actual temperature of the reaction mixture during the process was monitored and maintained by a Parr model 4843 proportional controller with a sensor in the reaction vessel. Samples were collected through a sampling valve at 10 min intervals for 60 min after the reactor reached the desired processing temperature. Samples were also collected during come-up times for the temperature treatments of 175 (at 30 min), 200 (at 30 and 35 min), and 225 °C (at 30, 35, and 40 min) because of the measurable reduction of zearalenone. Collected samples were immediately placed in an ice bath and then stored at −20 °C until analysis. Temperatures of the buffer solution in the vessel were recorded every minute during the entire processing period to ensure reproducibility of the experiments.

Detection of Zearalenone by HPLC. The concentrations of zearalenone in the buffer solution before and after processing were determined by high-performance liquid chromatography (HPLC) as previously described (Ryu et al., 1999). Samples were injected directly into the HPLC following filtration (Supor, Gelman Science Inc., Ann Arbor, MI). The HPLC instrumentation consisted of a model 510 HPLC pump (Waters Corporation, Milford, MA), a model E60 injector (Valco Instruments Co., Inc., Houston, TX) coupled with a model 728 autosampler (Micromeritics, Norcross, GA), and a model 474 scanning fluorescence detector (Waters Corporation, Milford, MA). The wavelengths of the detector were set at 274 nm excitation and at 440 nm emission cutoff. A 150 mm × 3.9 mm i.d., 4 μm Nova-Pak C18 reversed-phase column (Waters Corporation, Milford, MA) and a Nova-Pak C18 guard column (Waters Corporation, Milford, MA) were used for chromatographic separations. A mobile phase consisting of methanol−acetonitrile−water (1:2:3) was used at a flow rate of 1 mL/min. All chromatographic separations were monitored with computer-controlled Millennium 2010 software (Waters Corporation, Milford, MA) connected to the HPLC instruments.

Calculation of Kinetics. The rate of destruction of zearalenone (R_Z) was expressed by the equation

$$R_Z = \mathrm{d}(C_R)/\mathrm{d}t = -kC_R$$

where C_R is the remaining concentration of zearalenone (μg/mL) at time t (min) and k is the reaction rate constant (min^{-1}). An equation to describe the first-order reaction was obtained by integration,

$$\ln(C_R) = \ln(C_0) - kt$$

where C_0 and C_R refer to the concentration (μg/mL) of initial and the remaining zearalenone after processing time t (min), respectively. The initial concentration (C_0) was taken to be the concentration of zearalenone when the desired processing temperature was reached. Processing time was plotted with respect to the $\ln(C_R)$, and the reaction constant was calculated from the slope of the rate law equation. The half-life was calculated from the rate law equation by allowing C_R to equal $0.5C_0$.

Statistical Analysis. The entire experiment was replicated three times, and the data obtained from this experiment were analyzed by the Statistical Analysis System ($\alpha = 0.05$, SAS Institute, Cary, NC). Regression analysis on the natural log of zearalenone concentrations that accounted for the effects of time and replication was performed for each pH and temperature combination to determine reaction constants. The coefficient of determination was used to measure the fit of the regressions, and the half-life was estimated using the estimated regression coefficient. Regression analyses were not conducted at 100 and 225 °C because there was minimal destruction at 100 °C and complete destruction at 225 °C. The mean values and standard deviations were calculated with Excel (Microsoft Corp., Seattle, WA).

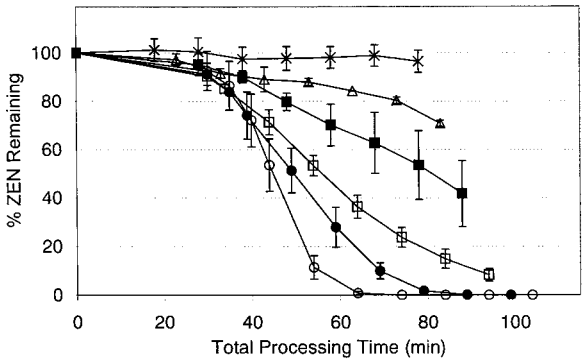

Figure 2. Effects of thermal processing on the reduction of zearalenone in an aqueous buffer at pH 4: zearalenone remaining in solution when heated at 100 (asterisk), 125 (open triangle), 150 (closed square), 175 (open square), 200 (closed circle), and 225 °C (open circle). Each point represents the average of three replicates, and error bars indicate one standard deviation from the mean.

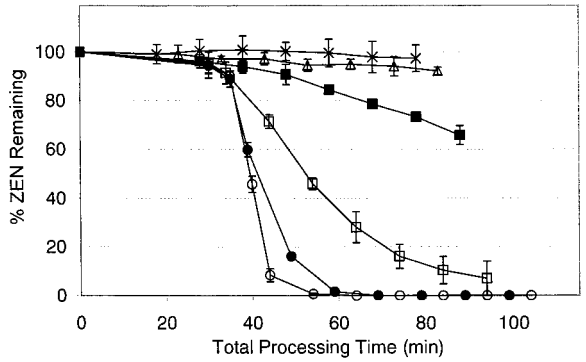

Figure 3. Effects of thermal processing on the reduction of zearalenone in an aqueous buffer at pH 7: zearalenone remaining in solution when heated at 100 (asterisk), 125 (open triangle), 150 (closed square), 175 (open square), 200 (closed circle), and 225 °C (open circle). Each point represents the average of three replicates, and error bars indicate one standard deviation from the mean.

RESULTS AND DISCUSSION

Reproducibility of Heat Processing. Actual temperatures of the solution during come-up and processing times were monitored to ensure reproducibility. Come-up time refers to the length of time necessary for zearalenone solutions to reach the desired processing temperatures. The come-up times to reach 100, 125, 150, 175, 200, and 225 °C were 18, 23, 28, 34, 39, and 44 min, respectively. The variation in temperatures at each processing time was ±2 °C during the come-up time and ±1 °C after the processing temperature was reached.

Effect of pH, Temperature, and Time on Destruction of Zearalenone. Destruction of zearalenone during thermal processing depended on the pH of the solution (**Figures 2−4**). The total processing time in each figure includes different come-up times (18−44 min) for each different processing temperature. Therefore, figures also show reductions of zearalenone during the come-up time if there are any. At processing temperatures less than 200 °C, loss of zearalenone was most rapid and extensive at pH 10, followed by that at pH 4 and 7. In contrast, the pH had no effect on the rate of destruction of zearalenone at 200 and 225 °C. After 60 min of processing at 200 and 225 °C, complete destruction of zearalenone was achieved at each

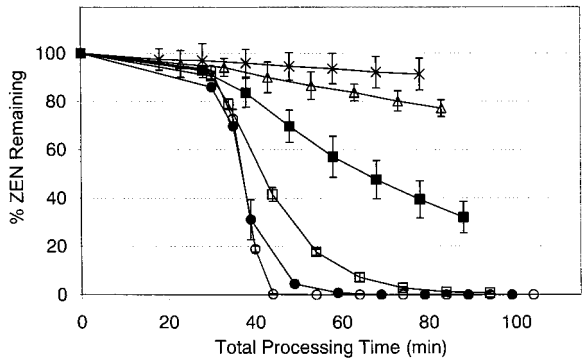

Figure 4. Effects of thermal processing on the reduction of zearalenone in an aqueous buffer at pH 10: zearalenone remaining in solution when heated at 100 (asterisk), 125 (open triangle), 150 (closed square), 175 (open square), 200 (closed circle), and 225 °C (open circle). Each point represents the average of three replicates, and error bars indicate one standard deviation from the mean.

pH. Overall, zearalenone appeared to be the least stable at pH 10 and the most stable at pH 7. It should be noted, however, that greater destruction of zearalenone was achieved at pH 4 than at pH 7 at temperatures of ≥175 °C.

Figures 2−4 also demonstrate that the rate of destruction of zearalenone was highly dependent on temperature. In general, the extent of destruction increased with processing temperature and time. Regardless of pH, no significant losses in zearalenone occurred during processing at 100 °C, and 8−29% of zearalenone was lost at 125 °C. In comparison, destruction of zearalenone reached 34% at pH 7 and 68% at pH 4 and pH 10 after 60 min of processing at 150 °C. At temperatures of ≥175 °C, greater than 90% of zearalenone was lost after the 60 min processing, and complete reduction of zearalenone was observed in less than 30 min at 225 °C, regardless of pH.

The results shown here are generally in agreement with the results of previous studies on the thermal stability of zearalenone. No change was observed in the amount of zearalenone after 4 h of heating in an aqueous solution at 120 °C (*9*). In another study (*5*), zearalenone content in ground corn was not changed by heating at 150 °C for 44 h. In comparison, 34% reduction in zearalenone concentration was observed by heating at 150 °C for 1 h at neutral pH in our experiment. This difference could be attributed to the absence of food matrix, e.g., ground corn, and possibly pressure-generated in the vessel during heating. When spiked into wheat flour (20 μg zearalenone/g) containing water (35%), zearalenone levels were reduced by 3.2% after heating for 15 min at 100 °C and 28.5% after 60 min at 150 °C, while at 200 °C, the amount of toxin was decreased linearly with heating time by 37% and 69% after 30 and 60 min, respectively (*6*).

Kinetics of Zearalenone Destruction. The destruction of zearalenone in buffers (pH 4, 7, and 10) heated at 125, 150, 175, and 200 °C followed first-order reaction. Linear correlation coefficients (k) demonstrate the straight-line relationship between processing time and the natural log of the fraction of zearalenone remaining. Half-lives ($t_{1/2}$) and first-order reaction constants indicate that the greatest destruction of zearalenone occurred at 200 °C with substantial reduction during the come-up time (**Figures 2−4**). In general, the destruction of zearalenone was fastest at pH 10 and slowest at pH 7 at temperatures below 175 °C. However, the rate of destruction of zearalenone was greater at pH 7 than at pH 4 at temperatures above 175 °C.

This is the first systematic study of the thermal stability of zearalenone in the absence of a food matrix. Therefore, possibilities such as binding with food matrixes or analytical problems were eliminated to estimate the true losses of zearalenone during the heat processing. These data confirm that zearalenone is very heat-stable, especially at neutral pH. In general, the loss of zearalenone was more rapid and extensive under alkaline conditions than at neutral or acidic pH, but temperature and processing time were more critical factors that affected the destruction of zearalenone. These results suggest that little or no change in zearalenone content would be expected when foods are heated at boiling or retort temperatures, i.e., 100−125 °C. However, substantial reduction of zearalenone may be achieved in food processes such as baking, frying, and extrusion where temperatures may reach or exceed 150 °C. It should be noted that although loss of zearalenone was shown by HPLC, this may not mean detoxification. More research is needed to fully assess the effects of thermal processes in reducing the biological activity of zearalenone and to prove loss of toxicity.

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