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Effects of Extrusion Temperature and Dwell Time on Aflatoxin Levels in Cottonseed

MICHAEL D. BUSER*,† AND HAMED K. ABBAS‡

Cotton Ginning Research Unit and Crop Genetics and Production Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Stoneville, Mississippi 38776

Cottonseed is an economical source of protein and is commonly used in balancing livestock rations; however, its use is typically limited by protein, fat, gossypol, and aflatoxin contents. Whole cottonseed was extruded to determine if the temperature and dwell time (multiple stages of processing) associated with the process affected aflatoxin levels. The extrusion temperature study showed that aflatoxin levels were reduced by an additional 33% when the cottonseed was extruded at 160 °C as compared to 104 °C. Furthermore, the multiple-pass extrusion study indicated that aflatoxin levels were reduced by an additional 55% when the cottonseed was extruded four times as compared to one time. To estimate the aflatoxin reductions due to extrusion temperature and dwell time, the least mean fits obtained for the individual studies were combined. Total estimated reductions of 55% (three stages of processing at 104 °C), 50% (two stages of processing at 132 °C), and 47% (one stage of processing at 160 °C) were obtained from the combined equations. If the extreme conditions (four stages of processing at 160 °C) of the evaluation studies are applied to the combined temperature and processing equation, the resulting aflatoxin reduction would be 76%.

KEYWORDS: Cottonseed; *Aspergillus flavus*; extrusion; aflatoxin; processing

INTRODUCTION

Cotton gins nationwide produce ∼7.7 million metric tons of cottonseed annually, of which ∼1.7 million metric tons of whole cottonseed is fed to livestock. Cottonseed meal is a coproduct of the cottonseed oil extraction industry, and an estimated 1.4 million metric tons is utilized in livestock rations nationwide. Cottonseed (whole) and cottonseed meal provide a good supply of protein, fat, and fiber in livestock rations. However, these products have been known to contain aflatoxin, which is of concern for animal and human health.

Aflatoxins, secondary metabolites of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, are acute toxins to most animals and are the most hepatoxic and hepatocarcinogenic natural agents known (*1*−*4*). *A. flavus* is universal and propagates in any substratum capable of supporting fungal growth, especially in warm humid environments (*5*). These fungi can infect crops before and after harvest and produce aflatoxins, thereby contaminating foods and feeds (*6*, *7*). Because the production of aflatoxin is so dependent upon environmental conditions, the amount actually produced varies widely from sample to sample and from year to year. The U.S. Food and Drug Administration's (FDA) aflatoxin action level for all foods containing cottonseed or cottonseed meal, including animal feeds, is 300 ppb for beef cattle, swine, and poultry and 20 ppb for dairy animals, animal species or uses not previously specified, or when the intended use is not known.

Aflatoxin detection test results are inherently variable and can be attributed to sampling, subsampling, and analytical variability. Whitaker et al. (*8*−*10*) indicated that sampling variability, especially for small sample sizes, is the largest source of error in determining aflatoxin concentration. Variability associated with sampling is primarily due to the extreme range of aflatoxin concentrations among individual seeds in a contaminated lot, resulting in a large variation among replicated samples. Subsampling and analytical variability are relatively minor in comparison to sampling variability and can be limited with proper laboratory practices.

There are several proposed methods of processing cottonseed (whole) to reduce aflatoxin levels. Ammoniation is a relatively common process used in Arizona and California; however, the process is not an FDA-approved practice, so the ammoniated material must be used on-farm or sold for use within the state. With proper treatment, the process has been shown to reduce aflatoxin concentrations by ∼95%. Blending is another alternative that has been used to reduce moderate concentrations of aflatoxin. The FDA does not permit the blending of contaminated and uncontaminated commodities but does allow the mixing of different levels of contaminated commodities. Irrespective of the processing method, the final product must be retested, fall within the regulatory limits, and be properly labeled. The literature on ammoniation and blending is quite

* Address correspondence to this author at 111 Experiment Station Road, P.O. Box 256, Stoneville, MS 38776 [telephone (662) 686-3093; fax (662) 686-5483; e-mail mbuser@ars.usda.gov].
† Cotton Ginning Research Unit.
‡ Crop Genetics and Production Research Unit.

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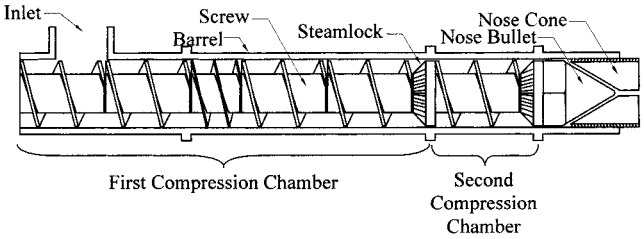

Figure 1. Extruder barrel cross section.

clear on the effectiveness of the processes, but there are several conflicting reports on the reduction of aflatoxin levels in cottonseed (whole) due to cooking, extruding, or, in general terms, processes that utilized relatively high temperatures and pressures. For example, Fischback and Campbell (*11*) reported that it was necessary to raise the temperature to ≥300 °C to decompose aflatoxins, and even then reductions were limited. Goldblatt (*12*) stated that a temperature of 100 °C decreased aflatoxin concentrations.

A recent report by Kenkel and Anderson (*13*) suggests that roasting temperatures of 143−149 °C can reduce aflatoxin levels by 40−50% in corn. Very limited recent information is available in the literature on the effects of temperature and pressure on aflatoxin levels. However, there is information in the literature on how these parameters affect other mycotoxin levels. Katta et al. (*14*) suggests that an extrusion process with temperatures in the range of 160−200 °C and screw speeds ranging from 120 to 160 rpm will reduce fumonisin B_1 by 46−76%. Castelo et al. (*15*) reports that greater fumonisin B_1 reductions were obtained at an extrusion temperature of 120 °C as compared to 140 °C. Ryu et al. (*16*) focused on zearalenone and reported greater reductions at an extrusion temperature of 120 or 140 °C as compared to 160 °C. They further suggested that reductions of 77−83% with mixing screws and 73−77% without mixing screws were obtained at an extrusion temperature of 120 °C. Some studies have included the processing time and/or extruder configuration associated with the experimental tests; however, there were no studies found describing the effects of dwell time on mycotoxin reductions.

The objective of this study was to determine the reductions of aflatoxin levels in cottonseed (whole) due to the extrusion process. The extrusion process was evaluated in terms of material temperature and dwell time. In an industrial setting, dwell time can be adjusted by varying the screw pitch (i.e., using screw segments of differing pitches), adjusting screw speed, adjusting the number of compression chambers, addition of lubricants, etc. Due to the number of factors that can effect dwell time, a simplified means of evaluating dwell time was required. Therefore, dwell time was evaluated by extruding the material multiple times with the same extruder configuration.

MATERIALS AND METHODS

Extrusion Equipment. The commercial-size extruding machinery at the Insta-Pro International Research and Development Facility in Des Moines, IA, was used for this study. The machinery consisted of an Insta-Pro model 2500 dry-extruder followed by an Insta-Pro air-type belt dryer to cool the material. This extruder is a single-screw adiabatic extruder that generates heat through friction. It is commonly referred to as a high-temperature, short-time extruder, which can achieve temperatures up to 180 °C in <20 s. The inside diameter of the barrel is 16.5 cm, and the overall length is 107 cm, with a constant-diameter screw. The barrel was configured with two compression chambers for the purposes of this study. A schematic of the extruder barrel is shown in **Figure 1**. The pitch of the worm flights determines compression. Shear is determined by the size of the steamlocks, the screw flight, and the adjustment of the nose bullet and cone in the last chamber of the barrel. The barrel wall and steamlocks are grooved to enhance mixing and shearing of the product being extruded (*17*).

The material was fed into the extruder through an electronically controlled volumetric feeder equipped with an agitator, which provided a relatively uniform and free-flowing material. Once the material entered the inlet chamber, it was forced into the first steamlock by the screw. Grooves in the steamlock walls allowed for a gradual buildup in pressure as the material passed through the compression chambers. When the material reached the last chamber containing the nose bullet and cone, an estimated maximum pressure of 2750 kPa had been achieved.

Extrusion is a process that applies pressure and shear to the material being extruded. In addition, the material is being internally mixed, to create a more uniform final material. The mixing process, along with pressure and shear, produces frictional forces between the material particles and between the particles and the internal barrel components, thereby heating the product being extruded. These characteristics of extrusion are dependent on one another. Therefore, these characteristics will be lumped together and defined as the extrusion process, in terms of extruder temperature.

Sampling and Lot Preparation. Due to the amount of manual material handling, time, and resources required for these replicated tests, relatively small lot sizes were essential. A key for determining lot sizes was the required amount of sample needed for the aflatoxin analysis. The sampling procedures were generally based on the *FDA Office of Regulatory Affairs Inspectional References: Investigations Operations Manual's* guide for mycotoxin sample size, which are used to obtain representative aflatoxin analysis for truckloads or other large quantities of material. A sample size of 1−1.5 kg (the FDA's operation manual suggests an individual sample size of 0.5 kg when the material has initially tested positive for aflatoxin) was selected for this study because the material used for these tests had initially tested positive for high levels of aflatoxin. The entire amount of contaminated material used was mixed before and during lot preparation; 15−20 random subsamples were combined for each sample. The tests were replicated, and analyses were confirmed by a secondary laboratory. Lot sizes for the extrusion temperature and multiple-pass extrusion tests were 70 and 140 kg, respectively.

The moisture content of the cottonseed used in the study was ∼6.5%; therefore, tap water was injected into the compression chambers as a lubricant to enhance the material flow uniformity through the extruder. The water injection rates were relatively low. For example, the highest rate used was 30 L/h, and the material production rate was ∼900 kg/h; therefore, 2.3 L of water was injected for the entire 70 kg lot. Because the extruder barrel is a closed system, the injected water is mixed with the cottonseed, thereby increasing the moisture content of the cottonseed. There is no runoff or aqueous environment created by injecting water into the extruder under these conditions. However, some of the added water is volatilized when the heated cottonseed (extruded) exits the extruder in an ambient environment.

After the material in the temperature study had been processed, the entire extruded lot was placed on a large piece of cardboard and the material was spread out uniformly before the subsamples were collected. During the multiple-pass study, the extruded material was collected in several plastic tubs, where subsamples were collected throughout the extruded material, before the material was reprocessed. A minimum of 15 random subsamples were collected and mixed together to produce one sample. Throughout the remainder of this paper, the word "sample" refers to a collection of subsamples, which were collected as previously discussed. After each test treatment, four random samples were collected for postprocessing aflatoxin analyses. Three of these samples were analyzed by one laboratory, and a second laboratory analyzed the other sample.

Each sample was ground and well mixed. Fifty-gram subsamples were taken from each sample and extracted with 70% methanol in water at a 1:5 w/v ratio, respectively. Each subsample was extracted in a blender at high speed for 3 min. The extract was separated in a centrifuge at 10000 rpm for 10 min. An aliquot of the supernatant was taken and used for determination of aflatoxin levels using commercially

available kits from Neogen Inc., Lansing, MI. Abouzied et al. (*18*) described the analysis procedure in detail.

Statistical analyses of the aflatoxin measurements from both studies were based on completely randomized designs blocked by testing laboratory. Furthermore, measurements from the multiple-pass study were also blocked by replication. On the basis of initial data plots and residual analysis, it was determined that there were unequal variances between treatments. Therefore, the data were transformed and the statistical models were developed using the natural logarithm of the aflatoxin measurements. From an intuitive perspective, it was expected that if temperature and/or increased dwell time (increased passes) affected the aflatoxin measurements, then the defining models would be based on an exponential decay of the aflatoxin levels with increased temperature and/or dwell time and not a linear model. Statistical analyses were conducted to determine if the aflatoxin measurements made by the second laboratory confirmed the measurements made by the first laboratory (i.e., testing block effects). The nonhomogeneity (interaction between the laboratory and temperature) between measurements made at the two laboratories was not significant (F value = 0.00; p value = 0.9808). Similar results were obtained for the multiple-pass study (F value = 0.64; p value = 0.4373). Therefore, these analyses indicated that the measurements made by the second laboratory confirmed the measurements made by the first laboratory.

Extrusion Temperature. This study required 840 kg of contaminated cottonseed. The Chickasha Cotton Oil Co. (Casa Grande, AZ) supplied roughly 1850 kg of aflatoxin-contaminated cottonseed for use in this study and the multiple-pass extrusion study. The cottonseed was produced in Maricopa County, Arizona, and the cottonseed initially tested positive for aflatoxin with levels in excess of 650 ppb.

This test focused on extrusion temperatures of 104, 132, and 160 °C. These temperatures were based on preliminary extrusion tests, which indicated that aflatoxin levels and variability were reduced at temperatures in excess of 114 °C. During these tests, temperatures were maintained within ±1 °C of the target values. As the pressure and shear were increased, by adjusting the extruder nose cone (required to increase temperature), water injection rates had to be increased to keep the material flowing through the barrel. Water injection rates were 15, 19, and 30 L/h, and the extruder pulled an average current of 70, 80, and 86 A for temperatures of 104, 132, and 160 °C, respectively. Four replications were performed for each temperature treatment, requiring a total of 12 lots. Contaminated seed from the bulk supply, not used to generate the lots, was used before and between lots to adjust the extruder temperature to the proper levels.

Multiple-Pass Extrusion. This study required ~420 kg of aflatoxin-contaminated cottonseed drawn from the same bulk supply received from the Chickasha Cotton Oil Co. During this test, each lot was extruded four times with samples for aflatoxin analysis collected after each pass. Three replications were completed, requiring a total of three lots.

The target extrusion temperature for this test was 132 °C. Extrusion temperatures were maintained within the range of 132−138 °C. Water injection rates were maintained at 19 L/h, and the extruder current draw ranged from 72 to 78 A for all stages of processing. Numerous nose cone adjustments were required to maintain a relatively constant and uniform flow rate for all stages of processing. This was due to the changes in the physical makeup of the material, which was altered by each stage of processing. The method of processing was conducted in the following manner: a lot was extruded; the material was collected in plastic tubs; samples were randomly collected; the material was reprocessed by the extruder; and the process was repeated until the lot was processed four times. This method was repeated for each lot.

RESULTS AND DISCUSSION

Extrusion Temperature. Statistical analyses were conducted using a natural logarithm model of the aflatoxin measurements as a function of temperature, where the interactions due to laboratory and temperature were treated as random errors. The mean square error for the analysis was 0.1655 with 46 degrees of freedom. Means and 95% confidence intervals for temperatures of 104, 132, and 160 °C were 182 ± 44, 137 ± 34, and 117 ± 21 ppb, respectively. On the basis of the analysis, there was a significant (0.05 level) reduction in aflatoxin levels as the temperature increased (F value = 7.81; p value = 0.0075). The least-squares means fit, means, and 95% confidence intervals are shown in **Figure 2**. The slope associated with the least-squares means fit indicates that the aflatoxin levels were reduced by an additional 33% when the cottonseed was extruded at 160 °C as compared to 104 °C.

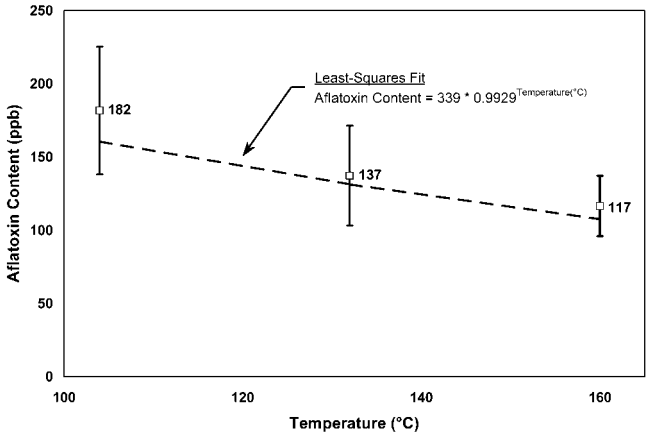

Figure 2. Aflatoxin means (squares), 95% confidence intervals (bars), and least-squares fit (line) for the aflatoxin study, conducted at various extruder temperatures.

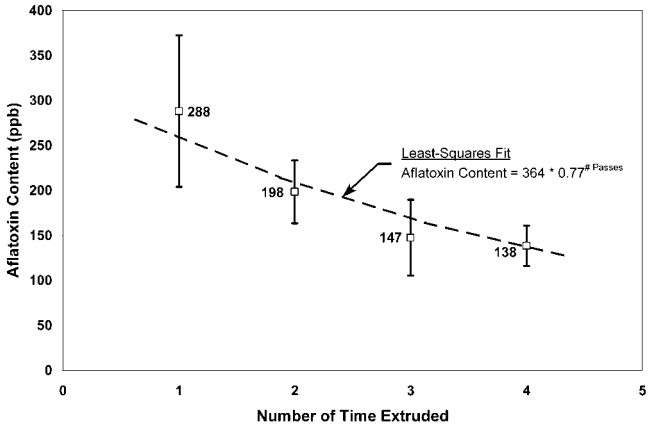

Figure 3. Aflatoxin means (squares), 95% confidence intervals (bars), and least-squares fit (line) for the multiple-pass aflatoxin study.

Multiple-Pass Extrusion. Statistical analyses were conducted using a natural logarithm model of the aflatoxin measurements as a function of the number of passes, where the interactions due to laboratory, replication, and number of passes were treated as random errors. The mean square error for the analysis was 0.1390. Means and 95% confidence intervals for one, two, three, and four passes were 288 ± 84, 198 ± 35, 147 ± 42, and 138 ± 22 ppb, respectively. On the basis of the analysis, there was a significant (0.05 level) reduction in aflatoxin levels as the number of passes increased (F value = 12.69; p value = 0.0063). The least-squares means fit, means, and 95% confidence intervals are shown in **Figure 3**. The slope associated with the least-squares means fit indicates that the aflatoxin levels were reduced by an additional 55% when the cottonseed was extruded four times as compared to one time. To reiterate, extruding the material multiple times was a simplified means of testing the effects of material dwell time. Results from this test should not imply that extruding the material multiple times is economically feasible; however, the tests do indicate that the

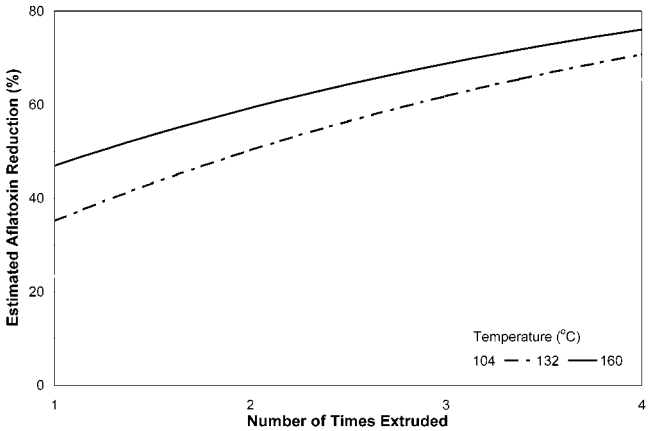

Figure 4. Estimated aflatoxin reductions based on the combined least-squares fits obtained from the temperature and multiple-pass studies.

extruder configuration will influence the amount of aflatoxin reduction obtained from the process.

The results from the temperature study are consistent with work reported by Goldblatt (*12*). However, the results are inconsistent with work reported by Fischback and Campbell (*11*), which implied that only limited aflatoxin reductions would occur if the toxin were subjected to temperatures in excess of 300 °C. To estimate the aflatoxin reductions due to extrusion temperature and dwell time, the least mean fits obtained for the individual studies were combined, as shown in **Figure 4**. Total estimated reductions of 55% (three stages of processing at 104 °C), 50% (two stages of processing at 132 °C), and 47% (one stage of processing at 160 °C) were obtained from the combined equations. This information is similar to work reported by Kenkel and Anderson (*13*), which suggested that roasting temperatures of 143−149 °C reduced aflatoxin levels by 40−50% in corn. If the extreme conditions (four stages of processing at 160 °C) of the studies are applied to the combined temperature and processing equation, the resulting aflatoxin reduction would be 76%. This estimated reduction is well below the 95% reduction due to ammoniation reported by Gardner et al. (*19*).

On the basis of the results of this study, further research should be conducted to determine the optimum extruder parameters required to achieve the largest reductions in aflatoxin levels with regard to economic feasibility.

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