Effect of high pressure ammoniation procedure on the detoxification of aflatoxins

Gomaa, M.N.E.; Ayesh, A.M.; Abdel Galil, M.M.; and Khayria Naguib

Food Science Department, National Research Centre, Dokki, Cairo, Egypt

Abstract

Ammoniation represents the best technique to detoxify aflatoxin-contaminated grain and it is considered as economically practicable for commercial applications. In the present study *Aspergillus parasiticus* was used to contaminate yellow corn to produce the final concentration reached 4000 µg/kg corn total aflatoxin. Two procedures of ammoniation (in aqueous ammonia concentrations, 0.25, 0.5, 1, 1.5 and 2%) were adopted for aflatoxin destruction. The first procedure was under atmospheric pressure at ambient temperature (AP/ AT) for 24 hrs, and the second procedure was under high pressure (2 bar) at high temperature (121°C) (HP/HT) for 15 min. Aflatoxin concentrations were determined by HPLC using fluorescence detection. The effect of HP/HT procedure was compared with the ammoniation procedure under AP/AT. The detoxification pattern of the two ammoniation procedures as well as the detoxification pattern of the different types of aflatoxins under the two procedures was studied.

Introduction

Every year a significant percentage of the world' s grain and oilseed crops is contaminated with hazardous mycotoxins including the aflatoxins. Unfortunately , discontinuing the feeding of aflatoxin-contaminated grain is not always practical, especially when alternative feedstuffs are not readily available or affordable (19).

Aflatoxins are potent hepatotoxins as well as potent carcinogens. The Food and Agriculture Organization (FAO) estimates that 25 % of the world' s food crops are affected by mycotoxins (10).

Significant aflatoxin contamination levels in corn and corn-based commodities have been reported in Latin America and the Caribbean. Aflatoxins were detected in many corn-based commodities such as corn, corn on cob, corn drink, Tortilla corn kernel, corn gluten raw, corn gluten feed, yellow corn, white corn, corn flour and flakes (16).

Sodium hydroxide, methylamine, hydrogen peroxide, ozone and other chemical reagents were used as inactivation treatments of aflatoxin. These chemical reagents achieved some degree of success, but generally were not economically practicable for commercial application (8, 16).

In the United States: Texas, North Carolina, Georgia, and Alabama have approved the ammoniation procedure for aflatoxin-contaminated corn. Mexico has approved

ammoniation for corn, also, many countries such as France, Brazil , Senegal , South Africa India and several countries of the European Economic Community use some ammonia-treated crops (17) .

The toxicity from ammonia aflatoxin reaction products was several orders of magnitude lower than that of aflatoxin B_1. Even the formation of these decontaminated reaction products in the feed matrix is usually < 1 % of the original aflatoxin contamination level. A large portion of the reaction products is bound to feed components such as protein and is potentially not biologically available to animals (15).

On the other hand Phillips et al. (20) reported that if the reaction between aflatoxin and ammonia is allowed to proceed sufficiently, the process is irreversible. The first step in the reaction is reversible, if the ammoniation process is carried out under mild conditions. However , when the reaction is allowed to proceed , the products formed do not revert back to aflatoxin B_1, also they added that the reaction products of ammoniation are dependent on temperature , pressure and the source of ammonia.

Human exposure to aflatoxins and other mycotoxins can result form direct consumption of contaminated commodities , or from the consumption of animal-derived foods. Therefore , our study aimed to compare between the effeciency of high pressure and atmospheric pressure ammoniation in the destruction of relatively high level of aflatoxins (4000 µg/kg) in contaminated yellow corn.

Materials and Methods

Aspergillus parasiticus NRRL 3 145 strain was subcultured on potato dextrose agar for 7 days at 25°C and stored at 4°C untill utilization. The previous fungal strain was activated on Potato Dextrose Agar (PDA) media which consists of 200g pealed potato, 20g dextrose and 15g Agar in 1 L distilled water.

Yellow corn was used as a model for an important component in different animal feeds which recorded frequent incidents of high levels of aflatoxin contamination.

Preparation of high concentration of aflatoxin contaminated corn

Yellow corn was artificially infected with the *Aspergillus parasiticus* strain according to Codner et al. (4) and Stubblefield et al. (24). The procedure can be describe as follows: 10ml water was added to 100g yellow corn into 1000ml Erlenmeyer flasks and the mixture was allowed to stand covered at room temperature overnight. The flasks were autoclaved at 121 °C for 20min, cooled and inoculated with 2.0ml of spore suspension which was prepared by adding 6.0ml of sterile water to a sporulated culture that had been incubated for at least seven days on Potato Dextrose Agar (PDA) at 25°C. Each inoculated flask was shaken every day and kept at 28°C for 15 days.

Preparation of final concentration of aflatoxin contaminated corn

The highly contaminated corn was diluted to the desired concentration by adding aflatoxin free corn. To ensure the homogeneity of sample both the contaminated corn and aflatoxin free corn were milled to the final particle size.

Ammoniation procedure for the 4000- µg-level aflatoxin

Two procedures of ammoniation were adopted for the destruction of 4000-µg-1evel aflatoxin. The main difference of the two procedures is the use of high pressure and temperature (HP/HT) along with ammonia for one procedure and using the ammonia under the atmospheric pressure and ambient temperature (AP / AT) , in the second one.

The moisture content of 40kg contaminated corn was adjusted to 18 % wet basis. Then ammonia was sprayed to provide a level of 0.25 , 0.5 , 1 .0, 1.5, and 2% ammonia on dry matter basis. Each ammonia concentration was used to spray 10kg contaminated corn to be used for the 2 ammoniation procedures (5kg each) .

a. Atmospheric pressure and ambient temperatures (AP/AT)
A total of 25 samples weighed 25 k g (5 samples of each ammonia concentration) were packed in polyethylene bags (lkg each) and stored for 24 hrs. The aflatoxin residues were determined by HPLC .

b. High pressure and high temperatures (HP/HT)
Another 25 contaminated corn samples (5 samples of each ammonia concentration) were packed in autoclavable polyethylene bags (lkg each) and autoclaved under high pressure (2 bar) at high temperature (121 °C). The corn was directly extracted to determine the aflatoxin residue by HJPLC.

Extraction and determination of aflatoxins
The extraction and clean up of aflatoxins in all samples were performed according to CB method (1) .

HPLC analysis
HPLC analysis was carried out with Waters Liquid Chromatography equipped with solvent delivery systems (model 6000A) , system controller (model 720), data module (model 730) , U6K injector and fluorescence detector (model 420) with excitation 338nm and emission 455nm. Econospher C 18 reverse phase column (5μ, 250mm XID 4.6mm) (Alltech) was used.

Derivatization
To the final extract (residue), an amount of 200μ1 hexane were added followed by 50μ1 triflouroacetic acid (TFA) and mixed well by a vortex shaker for exactly 30 sec.; the mixture was left to stand for 5 min. A mixture of 1.95 ml H_2O + acetonitrile (9+ 1 vIv) was added and mixed well for exactly 30 sec. and the mixture was left to stand for 10 min. Then the hexane layer was then discarded (19).

Preparation of aflatoxin standard
A different concentration of B_1(0.76 μM), B_2 (47.9 μM), G_1 (0.55 μM), and G_2 (0.75 μM) (Sigma Co.) were dissolved and mixed using methanol (HPLC grade).The methanol was then evaporated under a stream of nitrogen and the derivatization procedure was performed as previously described. The same derivatization procedure was applied on aflatoxin standard B_1, B_2, G_1 and G_2.

Chromatographic conditions
Mobile phases included solvent A [mixture of acetonitrile + water (23 + 77 v/v)] and solvent B (methanol). The linear gradient program was illustrated in Table (1).

LC determination
Only 20 μl of derivatized standard solutions was injected to prepare standard curve to check linearity of responses. A 20 μl of TF A-treated sample solution was injected. The aflatoxin concentration (μg/kg) of corn were calculated using standard curves for each toxin (B_1 , B_2, G_1 and G_2).

Table 1. The HPLC gradient program used for aflatoxin separation.

Time	Flow rate (ml/min)	% Solvent A	% Solvent B
0	1	100	0
5	1	60	40
10	1	40	60
15	1	0	100
20	1	100	0
25	1	100	0

Statistical analysis

The effect of different ammoniation treatments on the 4000µg aflatoxin contaminated corn was statistically analyzed using the two way analysis of variance. The significancy of differences between high and low pressure under different ammoniation level was tested according to the following model :

$$X_{ijk} = \mu + \alpha_i + B_j + \alpha_i B_j + E_{ijk}$$

where
- μ : General mean.
- X_{ijk} : Sample (K) of treatment (i) and concentration (j).
- α_i : Treatment (high & low) effect.
- B_j : NH_3 concentration effect.
- $\alpha_i B_j$: Interaction between pressure and concentration.
- E_{ijk} : Residual.

Main effect was used to detect the significancy of difference between each 2 treatments in the matrix of the different treatment levels. Regression analysis was performed to determine the slope and the regression order to identify the type and shape of the relationship between percent aflatoxin destruction and the 2 ammoniation treatments (high and low pressure) under different ammonia concentrations (23, 25).

Table 2. Effect of ammoniation treatment under low pressure on aflatoxins destruction.

Toxin	0.25%	0.5%	1%	1.5%	2%
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
G_1	58.74 ± 1.14^{a1}	78.76 ± 2.59^{b}	84.54 ± 1.20^{c}	94.44 ± 8.34^{d}	96.92 ± 1.30^{d}
B_1	39.52 ± 5.22^{a1}	66.94 ± 1.27^{b1}	77.44 ± 0.40^{c1d1}	80.74 ± 1.56^{d1}	88.02 ± 2.81^{d1}
G_2	29.70 ± 0.85^{a2}	74.80 ± 3.67^{b2}	83.90 ± 2.69^{c2}	89.40 ± 1.85^{c2d2}	93.02 ± 1.03^{d2}
B_2	34.52 ± 3.26^{a3}	63.18 ± 3.43^{b3}	73.14 ± 3.20^{b3c3}	78.72 ± 2.14^{c3d3}	85.40 ± 1.64^{d3}
Total	40.78 ± 2.43^{a4}	70.94 ± 2.71^{b4}	79.78 ± 1.81^{c4}	85.84 ± 1.19^{c4d4}	90.02 ± 1.36^{d4}

SE = Standard error
Values have the same letters are not segnificant

Results and Discussions

1. Effect of ammonia concentration on the stability of aflatoxins

a. Atmospheric pressure

Data presented in Table (2) showed the effect of ammonia concentrations on the stability of aflatoxins (G_1, B_1, G_2 and B_2) under atmospheric pressure. A proportional increase in destruction of aflatoxin was noted with the increase of ammonia concentrations (0.25, 0.5, 1.0, 1.5 and 2.0%).

This incline relationship was come to a plateau (no obvious increase in the aflatoxin percent destruction) with the use of 1.5 % ammonia concentration (Figure 1) . Regression analysis confirmed this relationship which was significant at the second order (Table 6). Statistical analysis revealed that significant differences were observed among the effects of 0.25, 0.5, and 1 .0 % ammonia concentration on aflatoxins destruction. On the other hand no significant differences were noticed between 1.5 and 2.0 % ammonia concentration for aflatoxins destruction.

The above mentioned results concerning the destruction of aflatoxin by different ammonia concentration under atmospheric pressure ranging from 40.8% (with 0.25% ammonia) to 90% (with 2.0 % ammonia) total aflatoxins percent destruction, was similar to those reported by Koltun et al., (8) who found that increasing ammonia concentration from 3% to 5% at 180°F for 15 minutes, increased total aflatoxins percent destruction from 45 % to 86 % . Similarly , Bagley (2) confirmed this relation when reported that aflatoxin B_1 percent destruction was increased from 83 % to 89% when ammonia concentration increased from 0.5 % to 1.5 % .

On the other hand, other investigators reported higher aflatoxin destruction when performing ammoniation under atmospheric pressure. In this concern, Jorgensen and Ralph (7) reported that 2% ammonia and 43°C for 15 days resulted in 98.8% aflatoxin B_1 destruction in naturally contaminated whole cottonseed.

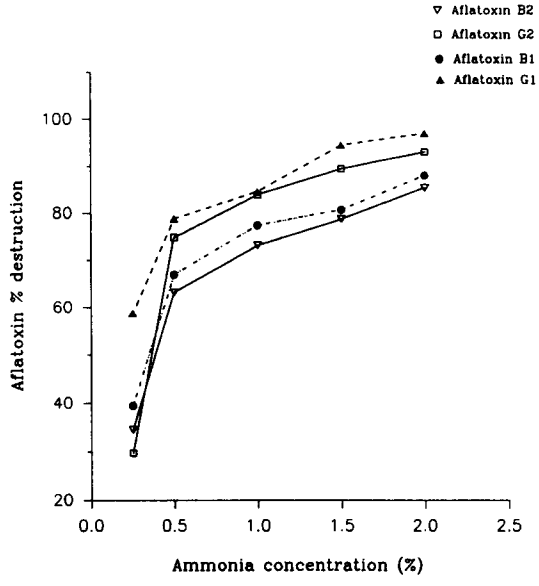

Fig. 1. Effect of different ammonia concentrations under atmospheric pressure on aflatoxins % destruction.

Table 3. Effect of ammoniation treatment under high pressure on aflatoxins destruction percentage.

Toxin	0.25%	0.5%	1%	1.5%	2%
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
G_1	87.14 ± 1.77^{a}	96.78 ± 1.09^{b}	99.54 ± 0.08^{b}	99.80 ± 0.06^{b}	100.0 ± 0.0^{b}
B_1	23.72 ± 5.18^{a1}	76.64 ± 5.32^{b1}	94.28 ± 1.00^{c1}	97.14 ± 0.25^{c1}	99.90 ± 0.08^{c1}
G_2	81.16 ± 3.15^{a2}	94.58 ± 0.96^{b2}	98.52 ± 0.55^{b2c2}	99.64 ± 0.09^{b2c2}	100.0 ± 0.00^{c2}
B_2	81.88 ± 2.42^{a3}	91.28 ± 0.63^{b3}	98.04 ± 0.37^{c3}	98.84 ± 0.11^{c3}	99.76 ± 0.09^{c3}
Total	68.90 ± 2.95^{a4}	93.08 ± 1.52^{b4}	97.74 ± 0.37^{c4}	98.64 ± 0.013^{c4}	99.92 ± 0.05^{c4}

Table 4. Effect of ammoniation pressure regardless ammonia concentration on aflatoxins destruction percentage.

Toxin	High	Low	P
G_1	96.65 ± 1.07	82.68 ± 2.84	0.0001
B_1	80.61 ± 5.72	70.53 ± 3.63	0.0002
G_2	94.78 ± 1.56	74.16 ± 4.79	0.0001
B_2	93.96 ± 1.45	66.99 ± 3.81	0.0001
Total	92.60 ± 2.32	73.63 ± 3.70	0.0001

Table 5. Effect of ammonia concentration regardless ammoniation pressure on aflatoxins destruction percentage.

Toxin	0.25%	0.5%	1%	1.5%	2%
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
G_1	72.94 ± 4.84^{a}	87.77 ± 3.29^{b}	92.04 ± 2.56^{c}	97.12 ± 0.91^{d}	98.46 ± 0.53^{d}
B_1	32.50 ± 4.45^{a1}	71.79 ± 3.05^{b1}	85.86 ± 2.85^{c1}	88.94 ± 2.75^{c1d1}	93.96 ± 2.38^{d1}
G_2	55.43 ± 8.72^{a2}	84.69 ± 3.75^{b2}	91.21 ± 2.77^{c2}	94.52 ± 1.92^{c2d2}	96.51 ± 1.26^{d2}
B_2	58.20 ± 8.12^{a3}	77.23 ± 4.96^{b3}	85.59 ± 4.41^{c3}	88.70 ± 3.50^{c3d3}	92.50 ± 2.51^{d3}
Total	53.28 ± 5.25^{a4}	82.01 ± 3.97^{b4}	88.76 ± 3.12^{c4}	92.24 ± 3.21^{c4d4}	95.37 ± 1.63^{d4}

Similarly, Norred (13) reported that atmospheric ammoniation of contaminated corn with 100 ppb total aflatoxins resulted in destruction of 99 % . Also Park et al. (18), reported that aflatoxin in corn was inactivated by more than 96 % by ammoniation procedure . Comparable results were reprted by Mahalingam et al., (9) who found that AP/ AT ammoniation treatment reduced the aflatoxin content from 35 µg/ g to a undetectable level.

In the same respect Phillips et al. (20), reported that 1-5 % ammonia under atmospheric pressure at ambient temperature for 14-42 days reduced the aflatoxin levels in corn to equal or below 20ppb .

b. High pressure

Table (3) showed that ammoniation under high pressure resulted in a similar trend in aflatoxin destruction with the increase of ammonia concentrations (0.25, 0.5, 1.0, 1.5 and 2.0%).

However , the incline relationship comes to a plateau with the use of 1.0% ammonia concentration (Figure 2). This relationship was confirmed by regression analysis which proved to be significant at the second order (Table 6).

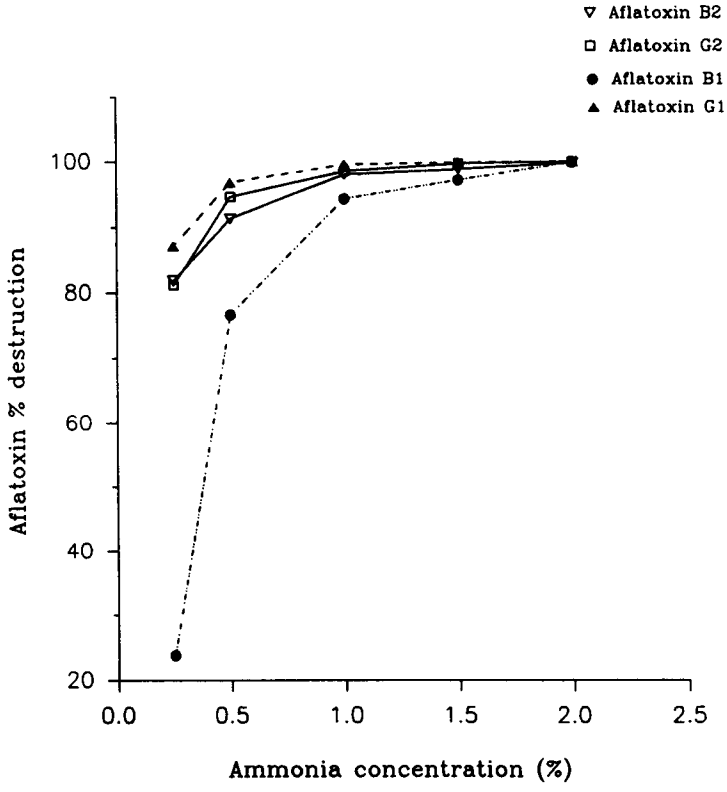

Fig. 2. Effect of different concentrations of ammonia under high pressure on aflatoxins % destruction.

Table 6. **Effect of different types of aflatoxins on the stability of aflatoxin under different treatment pressure.**

Type of toxin	Regression equation
	Atmospheric pressure
G_1	50.9 + NH_3 Conc 19.9 - NH_3 $Conc^2$ x 13.6
B_1	30.0 + NH_3 Conc 66.6 - NH_3 $Conc^2$ x 19.4
G_2	16.9 + NH_3 Conc103.2 - NH_3 $Conc^2$ x 33.2
B_2	24.5 + NH_3 Conc 69.2 - NH_3 $Conc^2$ x 19.9
Total	30.5 + NH_3 Conc 72.8 - NH_3 $Conc^2$ x 21.5
	High pressure
G_1	83.1 + NH_3 Conc 25.2 - NH_3 $Conc^2$ x 8.6
B_1	6.6 + NH_3 Conc131.4 - NH_3 $Conc^2$ x 43.4
G_2	74.6 + NH_3 Conc 33.0 - NH_3 $Conc^2$ x 10.5
B_2	75.1 + NH_3 Conc 32.9 - NH_3 $Conc^2$ x 10.5
Total	63.3 + NH_3 Conc 52.7 - NH_3 $Conc^2$ x 17.7

Statistical analysis revealed that significant differences were observed between aflatoxins destruction at 0.25 and 0.5% ammonia concentration. However, no significant differences were observed between 1.0, 1.5, and 2.0% .

In this respect Gardner et al. (6) noted that ammoniation of cottonseed meal under high pressure and temperature (250°F) reduced the levels of aflatoxin by more than 99%.

The obtained results were in agreement with those of Park et al. (14), who found that the treatment of contaminated meal (4000 μ g B_1 /kg) using 4% ammonia at 40 psi and 100°C for 30 minutes reduced the chemically detectable aflatoxin B_1 to less than 4 μg/kg (equals 99.9% destruction).

Similar results were reported by Samarajeewa et al. (20), who found that up to 5 % ammonia and 80-120°C or high pressure for 15-30 minutes reduced nearly completely aflatoxin in animal feeds. Phillips et al. (20), also found that 0.2-2% ammonia level under pressure 35-50 psi at 80-120°C for 2o-60 min reduced the aflatoxin concentrations in corn to equal or below 20ppb.

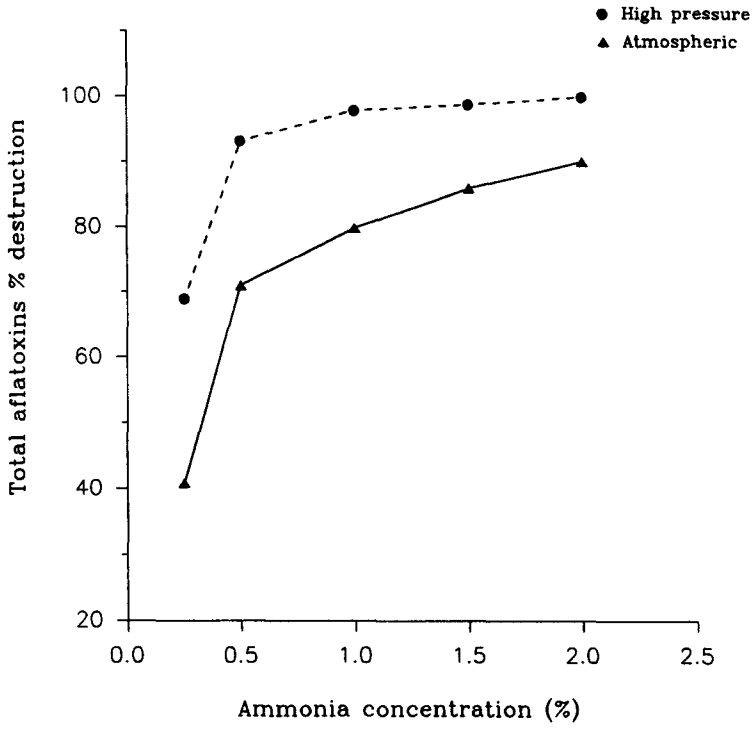

Fig. 3. Effect of different ammoniation pressures at different ammonia concentrations on total aflatoxins % destruction.

2. Effect of treatment pressure

Comparing Table (2) and Table (3) for the effect of pressure on aflatoxins destruction at the same ammonia concentration it becomes evident ammoniation under high pressure increase aflatoxins destruction for all types of tested aflatoxins except for aflatoxin B_1 at 0.25 % ammonia.

Regardless the ammonia concentration, Table (4) illustrated that the studied aflatoxins (G_1, B_1, G_2 and B_2) destruction percentage were higher under HP /HT treatment compared with treatment under AP/ AT.

The previous mentioned data in Table (4) was confirmed by the statistical analysis which proved a highly significant differences ($P > 0.001$) between high and low pressure for the tested aflatoxins .

In addition comparing the pattern of aflatoxin destruction under the high and atmospheric pressure (Figure 3) , showed that the effect of high pressure ammoniation was faster (plateau at 1 %) than atmospheric pressure ammoniation (plateau at 1.5 %) in reaching the maximum aflatoxins destruction.

Similar results were reported by Brekke et al. (3) who found that increasing temperature from 10°C to 40°C using 0.5% ammonia increased aflatoxin B1 destruction from 60% to 90% . Also Bagley (2) indicated that increasing temperature from 25°C to 60°C using 0.5% ammonia increased aflatoxin B_1 destruction from 75 % to 97% .

Confirming the above mentioned relationship between temperature and aflatoxin percent destruction, Mashaly et al. (11) found that 62% reduction in cottonseed

ammoniated for 7 days at 20°C was increased to 100% when using 100°C for 1 hour. Also Frayssinet (5) indicated that increasing pressure from 2 bar to 3 bar increased aflatoxin destruction from 86% to 94% .

3. Effect of different types of aflatoxins

Table (2) illustrated that under atmospheric pressure (AP/AT) aflatoxin B_2 showed higher stability against ammonia treatments (0.5 , 1 . 1.5 and 2 %) compared with the other types of aflatoxins. Figure (1) also confirmed this trend of the higher stability of group B compared with group G aflatoxins when ammoniated under atmospheric pressure.

The data in Table (3) indicated that treatment under high pressure and at the 0.25 % ammonia concentration B_1 recorded the lowest rate of destruction (23.7%) while G_1, G_2 and B_2 recorded higher destruction rate (more than 81%). Also at 0.5% ammonia B_1 was reduced by only 76% where more than 91 % of the other types of aflatoxins were desintegrated. Figure (2) illustrates that the higher stability of aflatoxin B_1 compared with the other types of aflatoxin was distinct at 0.25% and 50% ammonia concentration while at higher concentrations, these differences were getting closer.

Regardless of the treatment pressure (Table 5), aflatoxin B_1 recorded the minimum destruction rate (32.5 and 71.79%) at 0.25 and 0.50% ammonia. At the same time aflatoxins B_1 and B_2 were found to be more stable at 1.0, 1.5 and 2.0% ammonia compared with aflatoxins G_1 and G_2.

Regardless of the ammonia concentration, Table (4) indicated that aflatoxin B_2 is more stable (66.99% destruction) under low pressure treatment while aflatoxin B_1 is more stable (80.61 % destruction) under high pressure treatment. On the other hand, aflatoxin G_1 recorded the maximum destruction percent (82.68% and 96.65 %) at low and high pressure respectively.

The previous results indicating the higher stability of group B aflatoxins compared with group G aflatoxins were confirmed by Roegner (21) who reported that aflatoxins B was heat stable while aflatoxins G was heat labile. The higher stability of B_1 compared with B_2 was also reported by Moerck et al., (12) who treated naturally contaminated yellow corn containing 235 ppb of aflatoxin B_1 and B_2 with 0.5% aqueous NH_3 resulting in destruction of 60% for B_1 and 83% for B_2, respectively.

In general, the results revealed that the high pressure treatment was more destructive to aflatoxins than the treatment under atmospheric pressure. Moreover , high pressure ammoniation required minimum level of ammonia with less processing time .

The obtained results revealed that: 1. The effect of HP /HT procedure at the different ammonia concentrations was more destructive on aflatoxins than the AP / AT procedure. 2. Concerning the pattern of aflatoxin destruction, the effect of HP/HT was faster (plateau at 1%) than the AP/ AT (plateau at 1 .5 %) to come to the maximum aflatoxins destruction. 3. Aflatoxin B_2 showed higher stability for ammonia treatment (0.5, 1 , 1 .5 and 2 %) compared with the other types of aflatoxins. Also it was noticed the higher stability of group B compared with group G aflatoxin when ammoniated under AP/ AT. 4. Regardless treatment pressure, aflatoxin B_1 recorded the minimum destruction percent (32.5 and 71.79%) at 0.25 and 0.5% ammonia, respectively. At the same time, aflatoxins B_1 and B_2 were found to be more stable at 1.0, 1.5 and 2.0 % ammonia compared with aflatoxins G_1 and G_2. 5. Regardless ammonia concentration aflatoxin B_2 is more stable (66.99 % destruction under AP/ AT , while aflatoxin B_1 is more stable 80.61 % destruction) under HP/HT treatment. On the other hand aflatoxin G_1 recorded the maximum destruction percent (82.68 % and 96.65 % at low and high pressure, respectively.

References

1 Association of Official Analytical Chemists (AOAC) (1990). Official Methods of Analysis (15th Ed.), Washington, D.C.

2 Bagley , E.B. (1979). Detection of corn containing aflatoxin by treatment with ammonia. J. Am. Oil Chem. Soc . , 56: 808-811.

3 Brekke, O.L.; A.J. Peplinski and E.B. Lancaster (1977). Aflatoxin inactivation in corn by aqua ammonia. Trans. Amer. Soc. Agric. Eng., 20(6): 1160-1168.

4 Conder , R.C.; K. Sargeant and Yeo (1963). Production of aflatoxin by the culture of strains of Aspergillus-flavus-oryzae. on sterilized peanuts. Microbiological Biotechnology and Bioengineerings. 5: 185-192.

5 Frayssinet, C. (1990). Effect of ammoniation on the carcinogenicity of aflatoxin contaminated groundnut oil cakes. Long-term feeding study in the rat. Food Additives and contaminants 7(1) : 63-68.

6 Gardner , H.K. ; S.P. Koltun; F.G. Doller and E.T. Rayner (1971). Inactivation of aflatoxins in peanut and cotton seed meals by ammoniation. J. Am. Oil Chem. Soc., 48: 70-73.

7 Jorgensen, K.V. and L.P. Ralph (1981). Atmospheric pressure-ambient temperature reduction of aflatoxin B_1 in ammoniated cottonseed. J. Agric. Food Chem., 29:555-558.

8 Kolton, S.P.; E.T. Rayner. J.I. Wadsworth and H.K. Gardner JR. (1979). Inactivation of aflatoxins in cottonseed meal by ammoniation: 1. Reaction studies. J. Am. Oil Chem. Soc. 56(9): 803-807.

9 Mahalingam, R.; S. Gavindan; N. Punniamurthy and Balachandran, C., (1990). A study on aflatoxin detoxification by aqua-ammonia method in poultry feed. Indian Veterinery J. 67(2):149-151.

10 Mannon, J. and E. Johnson (1985). Fungi down on the farm. New scientist. 105(1446): 12-16.

11 Mashaly, R.I.; S.A. EI-Deeb; A.A. Ismil and A. Youssef (1983). Effect of some chemical treatments on detoxification of aflatoxin in cottonseed meals. Proceeding of Internal Symposium on Mycotoxin, Cairo, Egypt, 515.

12 Moerck, K.E.; P. McElfresh; A. Wohlman and B.W. Hilton (1980). Aflatoxin destruction in corn using sodium bisulfate, sodium hydroxide and aqeous ammonia. J. of Food Protection, 43(7): 571 5774.

13 Norred, W.P. (1982). Ammonia treatment to destroy aflatoxins in corn. J. of Food Protection, 45(10): 972-976.

14 Park, D.L.; L.S. Lee and S.A. Kolton (1984). Distribution of ammonia related aflatoxin reaction products in cottonseed meal. J. Am. Oil. Chem. Soc . , 61 : 1071 1074.

15 Park D.L. (1993). "Perspectives on mycotoxin decontamination procedures" Food Additives and contaminants. 10(1) : 49-60.

16 Park D.L. and Liang B. (1993): "Perspectives on aflatoxin control for human food and animal feed" Trends in Food Science & Technology , October 4: 334-243.

17 Park, D.L. and L.S. Lee (1990): "New perspectives on the ammonia treatment for decontamination ot. aflatoxins .In A persective on aflatoxin in field crops and animal food products in the United States" USDA/ARS ARS-83, June.

18 Park, D.L., L.S. Lee; R.L. Price and A.E. Pohland (1988): Review of the decontamination of aflatoxin by ammoniation : current status regulation. J. AOAC, 71 : 685-703.

19 Park, D.; S. Nesheim; M.W. Trucksess; M.E. Starck and R.F. Newell (1990). Liquid chromatographic method for determination of aflatoxins B_1, B_2, G_1 and G_2, in corn and peanut products. J.Assoc. off Anal. Chem. 73(2) workshop book, international symposium and workshop on food contamination mycotoxins and phycotoxins.
20 Phillips T.D., Clement B.A. and Park D.L. (1994). Approaches to reduction of aflatoxins in foods and feeds: the toxicology of aflatoxins: Human Health, Veterinary and agricultural significance, Copyright by Academic Press, Inc. USA, Part 111, p. 383-406.
21 Roegner, F.R. (1967). F.D.A. looks at aflatoxin. 38th Ann. Tech. Conf. Grain Elevators, Proc. Supl. Assn., Chigago, IL, Feb.
22 Samarajeewa, U . , A.C. Sen; M.D. Cohen and C.I. Wei (1990). Detoxification of aflatoxins in foods and feeds by physical and chemical methods. J. of Food Protection, 53(6): 489-501 .
23 SAS Institute (1990) SAS/STAT User's guide release 6.03 edition, SAS institute, Cary NC. U.S.A.
24 Stubblefield, R.D.: O.L. Shotwell: C.W. Hesseltine: M.L. Smith and H.H. Hall (1967). Production of aflatoxin on wheat and oats : Measurement with a recording densitometer. Applied Microbilogy, 15(1). 186-190.
25 Winer, B.J. (1971): Statistical principals in experimental design. 2nd ed. Chapter 7. 514-603, McGraw-Hill Kogahusha, LTD.

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