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ELIMINATION OF NATURALLY OCCURRING AFLATOXINS IN COTTONSEED MEAL

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Ammoniation appears to be one of the most extensively studied and applied chemical methods. The present study was aimed at determining optimum time period required at pilot plant scale to detoxify aflatoxins in cottonseed meal by ammoniation under steam pressure and elevated temperature. The detoxification was achieved upto 97% with one percent ammonia at 99 ± 1°C under 0.7± 0.035 bar steam pressure. The quality and texture of starting material was not significantly altered. This study was also aimed to develop a feasible process for detoxification of aflatoxins in cottonseed meal on commercial scale in Pakistan.

Key words: Aflatoxins, Detoxification, Ammonia, Cottonseed meal.

Introduction

Detoxification of mycotoxins, particularly of aflatoxins in animal feedstuffs, has an important position in research leading to various eradication methods which include physical removal of damaged/infested seeds or kernels, inactivation and degradation by biological or chemical means [1-3]. Although, aflatoxins appear to be the major issue, additional interest has also been directed in detoxification of other mycotoxins [1-3]. Various chemicals such as oxidants, alkali, acids and organic solvents have been used to either remove or convert aflatoxins into non-toxic forms [1-3]. Ammonia is one of the major alkali chemicals used in the detoxification of aflatoxins [4]. It has been reported that the major breakdown product of pure aflatoxin B_1 by ammoniation is aflatoxin D_1 with a molecular weight of 286, and others with molecular weights of 206 and 256 [4,5]. In the presence of meal matrix of agricultural commodities similar results have been reported but with lowered or eliminated conversion of aflatoxins to the compounds detected in model reactions [6,7]. Probably the formation of ammoniated aflatoxin by-products are influenced in the presence of meal matrix [7]. The ammoniation process marginally decreases the levels of sulfur containing amino acids, lysine, available lysine and non-reducing sugars, whereas, increases the level of total and non-protein nitrogen, ash and soluble solids [7]. Many reported studies support the use of ammoniation process for lowering the levels of aflatoxins in agricultural products [1-3,6,7].

Cottonseed meal is the most important and largest oilseed by-product of agricultural commodity in Pakistan. Unfortunately, its inclusion rate in poultry feed in Pakistan is restricted to 9% due to its contamination by mycotoxins, especially aflatoxins, and to some extent by increased gossypol content. Although the gossypol is found to be generally within the permissible limits in cottonseed meal the use of this commodity is generally low in poultry feed due to its potential side effects. The developed detoxification process not only destroys aflatoxins but also reduces the level of gossypol in the cottonseed meal (unpublished data).

The purpose of this study was to determine optimum conditions required for detoxification of contaminated cottonseed meal with minimum demand for ammonia, temperature and time. The research was also aimed at developing a process for commercial use in Pakistan. This process has also been patented in Pakistan [8].

Materials and Methods

Highly contaminated cottonseed meal was purchased from local grains wholesale market. Chemicals and reagents used in the analysis of aflatoxins were of analytical grade. The pilot plant for the ammoniation of aflatoxins and aflatoxins standards were kind gifts from Natural Resources Institute, Chatham, UK. Twenty-six batches of cottonseed meal (average 251 kg) were used in detoxification experiment with an average initial moisture content of 8.6±1.65% and contaminated aflatoxins (B_1+B_2) ranging from 128 µg/kg to 3322 µg/kg. Aflatoxins G_1 and G_2 were not detected in any of the samples.

The detoxification plant consisted of double walled stainless steel reaction vessel of approximately 0.5 ton capacity. Depending upon the bulk density of cottonseed meal an optimum load of 250 kg could be detoxified at any given time. Reaction vessel was provided with steam jacket for continuous heating. Known levels of ammonia and steam were introduced into the vessel after passing through respective regulators and scales. Reaction vessel containing cottonseed meal was preheated to 60°C prior to steam pressurization. Contents of the vessel were continuously agitated. Steam was introduced slowly into the vessel by opening the steam inlet control valve to raise the temperature up to 100° ± 2°C and pres-

sure up to 0.7 ± 0.035 bar within 10 min. The steam pressure and temperature were maintained to fulfill the water requirement of meal (15-18% moisture). Prior to introduction of ammonia, pressure of the vessel was reduced to prevent back-pressure and to maintain ammonia inlet-line pressure above the vessel pressure. Ammonia was introduced slowly through a flow-meter at a suitable driving pressure to provide ammonia (1.0%) within 5 min for a period of 25 min for the detoxification of cottonseed meal. At the end of the process, excess ammonia was removed under influx of pressurized steam with full opening of the vent-valve and exhaust system attached to the reaction vessel. The detoxified cottonseed meal was collected and spread on the floor in open air to cool it down. 50 kg samples of cottonseed meal prior to and after detoxification collected at respective collection points on the inlet and outlet conveyers, were further reduced to 10 kg on rotary sample divider for the analysis of aflatoxins. Remaining 40 kg was returned to the respective batch. Each analysis was done in triplicate. Water based slurry of 1 kg each of the sample was used [9] to detect aflatoxins by Romer clean up method (10, Section 26.081) followed by AOAC (10, Section 26.031) quantification method by comparisons of aflatoxins standards on thin layer chromatography plate (precoated silica gel 60) and confirmed by spraying with 50% sulphuric acid and making derivative with trifluoroacetic acid on the TLC plates [11]. Residual ammoniacal nitrogen was determined by the micro-Kjeldhal as described in the Methods of the AOAC (10, Section 7.038) on Tecator Kjeltec digestion and distillation units.

Results and Discussion

The ammoniation process under steam pressure has shown to reduce aflatoxin levels in cottonseed meal (Table 1) from the average content of 815 to 26 µg/kg, a reduction of 97.12% in aflatoxin content. This process, at an elevated temperature and low pressure has detoxified the cottonseed meal in 30 min. Also the texture and physical appearance of starting material was not significantly altered. The experimental trials conducted on broiler chicks for seven weeks with the poultry feed containing detoxified cottonseed meal upto inclusion rate of 15% , showed no determinental effects on poultry, indicating that ammonia treatment had effectively eliminated the aflatoxins. When compared with toxic feed containing 685 µg/kg of aflatoxin B_1 and control feed with 36 µg/kg of aflatoxins, the broiler chicks gained significantly better weight than when fed with the feed prepared with detoxified cottonseed meal. With detoxified feed, the average weight gain was 2.163 kg in comparison with toxic feed where the average weight gain was 1.768 kg while in control it was 1.948 kg. Feed conversion ratio was also better in case of detoxified feed in comparison with control and toxic feed [18].

Previous researches have demonstrated that compounds produced by ammoniation of aflatoxins in cottonseed meal in a model system or in the field are chemically and biologically far less toxic than the parent aflatoxins [3,7,13,14]. It has been reported that mutagenic activity, using Ames Test for the ammoia-related aflatoxin reaction products in cottonseed meal, was observed to be 2,000-20,000 times less than that observed with non-ammoniated aflatoxin B_1 [12].

Method of detoxification of aflatoxins by ammoniation, widely used in developed and developing countries [7,13], is particularly of two kinds: (a) atmospheric pressure ammoniation at ambient temperature where the product is sealed in plastic bags or bins for 2-3 weeks, and (b) high temperature and pressure ammonoation where treatment duration is less than an hour [3,7].

The States of Arizona and California in the USA, have now permitted the ammoniation of cottonseed products [7]. Cavanagh and Ensminger [15] outlined the nutritive and economic values of feeding ammoniated cottonseed meal to rumi-

TABLE 1. AFLATOXINS CONTENT AND MOISTURE (%) OF COTTONSEED MEAL BEFORE AND AFTER AMMONIATION.

Batch weight in kg	Aflatoxin content* µg/kg					% Detoxification	Moisture (%)	
	Before ammoniation			After ammoniation			Before	After
	B_1	B_2	Total	B_1	B_2			
250	500	70	570	25	nd	96.0	10.0	9.0
265	128	nd	128	nd	nd	99.9	10.5	9.5
250	228	nd	228	2	nd	99.0	9.1	9.1
262	758	nd	758	46	nd	94.0	11.1	9.5
250	654	nd	654	7	nd	99.0	6.8	8.6
255	179	51	230	2	nd	99.0	9.1	9.0
250	665	nd	655	53	nd	92.0	6.8	8.6
250	582	nd	582	16	nd	97.0	7.2	7.2
250	531	6	537	16	nd	97.0	7.2	7.2
250	549	5	564	16	nd	97.0	7.2	7.2
240	476	nd	476	22	nd	99.6	7.2	8.2
245	327	51	378	14	4	95.0	6.6	6.4
250	215	nd	215	4	nd	98.0	6.8	7.2
250	327	nd	327	24	nd	92.7	6.4	6.6
245	735	nd	768	27	nd	96.5	9.0	9.0
250	500	7	507	25	nd	95.0	10.0	9.0
250	128	nd	128	nd	nd	99.9	10.5	9.5
260	758	nd	758	15	nd	98.0	11.1	9.5
250	190	45	195	2	nd	99.0	9.1	9.0
275	659	nd	659	7	nd	99.0	6.8	8.6
248	3004	318	3322	122	6	96.0	10.5	9.5
250	2456	201	2657	46	3	98.0	9.6	9.4
247	1659	43	1702	57	nd	96.6	10.8	9.6
250	1011	nd	1011	18	nd	98.2	9.2	8.9
245	1273	57	1330	28	nd	97.8	7.3	7.1
250	1806	133	1839	39	nd	97.8	6.9	7.1
Avg =251.42	780.69	37.96	814.92	24.35	0.50	97.19	8.57	8.44
o^{-n} =7.083	704.90	73.14	763.80	25.38	1.45	2.07	1.62	1.03
o^{-n-1} = 6.95	718.92	74.59	778.90	25.88	1.48	2.11	1.65	1.05
n= 26	26	26	26	26	26	26	26	26

* Results from analysis of 3 replicates., nd= Not detected.

nants. Regulatory programme for control of aflatoxins in France utilizes the ammoniation process and effectively limits aflatoxin levels in animal and dairy feeds to <20 and <5 µg/kg, respectively [16].

Programme of decontamination of aflatoxins by ammoniation is strongly supported by the African Groundnut Council and its Member States [13,17]. Although ammonia has an advantage of being inexpensive, it poses a little threat to ruminant, metabolism and can be dissipated during drying [3]. Until any biological or chemical evidence shows potential risk posed by ammoniation process, there is an overwhelming support for it as an economical and practical method for detoxification of aflatoxins in agricultural commodities and animal feed in developing countries, particularly, in Pakistan.

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