US Open Food Science & Technology Journal
Vol. 1, No. 2, June 2014, pp. 1- 8
Available online athttp://arepub.com/Journals.php

Research Article

Quantifying Aflatoxins in Grain and Flour from the Five Largest Storage Facilities in Central Sudan Using AfalTest® HPLC Method

Esameldin Bashir Mohamed Kabbashi[1] and Salah Eldeen Abbas Ali[2]

[1]Food Research Center, Shambat, Khartoum North.

[2]National Chemical laboratories, Federal Ministry of Health, Khartoum.

Corresponding Author: esameldinkabbashi@gmail.com, Tel.: +249 (912236967/ 922226554 and 122034853), Fax: +249 -185 – 311049

Abstract

Aflatoxins are products of toxigenic *Aspegillus* spp. That is *Aspergillus flavus* produces B types (B1 and B2) whereas *Aspergillus parasiticus* produces both B and G types (G1 and G2). These mycotoxins are the most known potent hepatogenic chemical besides their carcinogenic, mutagenic and teratogenic effects. Also they cause a lot of health complications for human and his domestics *i.e.* they retard growth in children, and cause aflatoxicosis. This study focused on the use of AflaTest® HPLC column to check 25 samples for the four types of aflatoxins. Only two samples were found positive for B1 aflatoxin showing 2.45 and 5.87 µg/ kg. Both figures of aflatoxin B1 are below the aflatoxin maximum limit of the Codex Alimentarus and the latter is more than the maximum limit of the European Union(EU) in several foodstuffs (10 and 5 ppb, respectively). The findings of this study, 8% contamination and the detectable concentrations, being less than the maximum acceptable level by the Codex Alimentarius, are very encouraging in keeping up the management quality in these huge storage facilities and adopting this check – up method to protect the consumers, all stakeholders of concern and the environment from any negative consequences followed the contamination by these catastrophic chemicals and to conserve a healthy and economic product.

Keywords: Aflatoxins, grain, flour, AflaTest®, storage system, safety limits.

Introduction

The need to save seed for the next crop and/ or the desire to consume cereal – based foods all year round means that many grains are stored for some time prior to use. So a major constraint in storage is maintaining the biochemical quality of grains. This quality may include germination quality, nutritional quality and remain safe for consumption as well [1]. However, the quality of stored grain partly depends on the chemical and biochemical properties of the grain and the atmosphere of the store and both grain temperature and moisture have a major influence on these parameters. However, it is also important to know the moisture sorption isotherm (MSI) which has a relationship with moisture content that has a sigmoid shaped curve when graphed. This MSI (= a_w, water activity) allows analysts to determine the moisture content and *viceversa* [1]. Moreover, reducing a_w in the grain will inhibit the growth of mycotoxins producing fungi. However, if the atmosphere is quite humid this may be difficult to achieve. Alternatively some chemicals e.g. propionic acid may be required to inhibit fungal growth in atmospheres that are warm and humid or on grain that has high moisture content [2]. Aflatoxin contamination problems are minimized with management such as thorough grain cleaning, proper combine adjustment to reduce kernel damage, matching drying capacity to wet corn holding capacity, proper drying, removal of fines and broken kernels, proper grain cooling after drying, and sound storage practices [3]. Moulds were observed on rice with more than 14.4% moisture content (m. c.) at 25°C, but no mould was observed on rice with less than 12.8% (m. c.) [4]. The availability of moisture and relatively high temperature induces microbial growth that produces toxic metabolites such as mycotoxins and consequently reduces food safety [5]. A hot spot that developed *A. flavus* growth in a bin of corn in central Illinois during warm weather has been investigated for mycotoxins. High levels of aflatoxin (1,000-1,700 ppb) were detected in samples collected near the center of the hot spot that was defined by visible *A.flavus* sporulation. The location of the hot spot relative to an open window indicated the moisture necessary for mould growth, and aflatoxin formation could have come from rains blown through the window. Aflatoxin was not detected in samples collected furthest from the window. Zearalenone was detected in some of the samples collected, but it was not confined to any one part of the bin. The corn had been in the field an unusually long time before harvest because of cold and wet weather [6]. Aflatoxins can be undetected in the hot spots if the sampling is not taken in a correct way [7], [8] & [9]. However, masked mycotoxins could appear in the feed. They are chemically modified mycotoxin products of specific biochemical reactions in which mycotoxins bound to certain feed ingredients including glycosides, glucuronides, fatty acid esters and proteins. Consequently these masked mycotoxins are not detectable with conventional analytical methods *i.e.* no contamination detected due to mycotoxin bound form [10], [11] & [12]. These elusive toxins will affect the fed individual(s) after been freed [13] & [14]. Aflatoxins can be reduced in major crops by good agricultural practice (GAP) such as adequate raw spacing, irrigation, weed reduction, insect control, early harvest, and rapid drying [15]. GAP cannot reliably reduce aflatoxins especially in the developing countries. However, inspite of the little practical progress in breeding for aflatoxin resistance this practice is considered a long term measure since *A. flavus* is a commensal and not a pathogen in the crop. Biocontrol of mycotoxins was tried e.g. using of *Trichoderma* spp. against pathogenic fungi and use of competitive exclusion in crops by using nontoxigenic strains to compete with toxigenic ones [15]. This study aim at using some advanced technology in detecting and quantifying aflatoxins in the greatest storage firms of grain in Sudan using AflaTest® column of the Waters Incorporation, USA.

Material and Methods

AflaTest® underwent evaluation by the United States Department of Agriculture, Federal Grain Inspection Services (FGIS) for the detection of total aflatoxin (B1, B2, G1 and G2) for corn, corn bran, corn flour, corn germ meal, corn gluten feed, corn gluten meal, corn meal, corn/soy blend, flaking corn grits, condensed distillers solubles, dried distillers grain, dried distillers grain with solubles, milled rice, brown rice, rough rice, rice bran, popcorn, sorghum, soybeans and wheat. Under the authority of the United States Grain Standards Act, this test kit was found to meet or exceed all design and test performance criteria as defined in "Design Criteria and Test Performance Specifications for Quantitative Aflatoxin Test kits". This test kit is cited in the AOAC® Official Methods Program, as official method 991.31applicable for the determination of aflatoxin B1, B2, G1 and G2 both by fluorometry and HPLC analysis. AflaTest® has final action AOAC Official Method status [16]. The AflaTest® test method has the following steps [16]

1.0 HPLC Set up.

2.0.*SampleExtraction*:
2.1. Weigh 25g grain/ grain product sample with 5g salt (NaCl) and place in blender jar.
2.2. Add to jar 100 ml methanol: water (80:20).
2.3. Cover blender jar and blend at high speed for 1 minute.
2.4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3.0.*ExtractDilution*
3.1. Pipet or pour 10 ml filtered extract into a clean vessel.
3.2. Dilute extract with 40 ml of purified water. Mix well.
3.3. Filter dilute extract through glass microfibre filter into a clean vessel.
4.0. Column Chromatography
4.1. Pass 4 ml of filtered diluted extract (4 ml = 0.2g sample equivalent) completely through AflaTest affinity column at a rate of about 1-2 drops/ second until air comes through column.
4.2. Pass 10 ml of methanol: water (20:80) through the column at a rate of about 2 drops/ second.
4.3. Repeat step 4.2. once more until air comes through column.
4.4. Place glass cuvette (VICAM part # 34000) under AflaTest column and add 1.0 mL HPLC grade methanol into glass syringe barrel. In this test clean glass flask was used instead.
4.5.E lute AflaTes t® column at a rate of 1 drop/ second by passing the methanol through the column and collecting all of the sample eluate (1.0 mL) in a glass cuvette. In this test clean glass flask used instead.
4.6. Add 1.0 ml of purified water to eluate. Inject 20 – 100 µl onto HPLC (Shimadzu®).
Note: For greater sensitivity, more sample volume can be passed over the column in step 4.1.

5.0. Recovery: 76% at 20 ppb (7B1:1B2:3G1:1G2 aflatoxin mix).

6. Results of the aflatoxin were found by calculating the injected concentration of aflatoxin divided by the area of the standard in the chromatogram and multiplied the area of the sample. The results reflect that only two samples were contaminated with aflatoxins B_1. The former has a score of 2.45 µg/ Kg (ppb) and the later 5.87 µg/ Kg (ppb).

Results and Discussion

The results of this experiment reflect that only two of the 25 samples tested were found to have an aflatoxin contamination within the detection limit (0.05 – 0.1 ppb) and quantification limit (0.1 – 0.15 ppb) of aflatoxins [17]. That is, the first contaminated sample had 2.45 µg/ Kg (ppb) and the next has 5.87 µg/ Kg (ppb) (Table, 1). However, the detection limit for aflatoxins was reported to be 10 ng whereas the corresponding quantification limit was 0.3 ng [18]. Figure 1 is the semolina sample chromatogram which displays a spike that has a retention time 3.381 minutes and figure 2 for the details of the standard including a spike with 3.885 minute retention time which is for aflatoxin B1the difference between the two retention time results is 0.004 minute which is very negligible and reflect the accuracy of the experiment and the HPLC device as well. It's clear that B1 aflatoxin is the reported form in the two figures (the spikes B1 in Fig. 1 and Fig. 2, respectively). The reported concentrations in the two samples 2.45 and 5.87 µg/ Kg (ppb) in sorghum and a semolina flour, respectively are both below the maximum limit of the Codex Alimentarius [10 µg/ Kg (ppb)] in peanuts, almonds, shelled Brazil nuts, hazelnuts and pistachios intended for further processing whereas it is 15 µg/ Kg (ppb) in almonds, hazelnuts, pistachios and shelled Brazil nuts (ready to eat)[19]. Nevertheless the World Bank issued a report in 2001 describing a study on the impact of adopting international food safety standards as harmonization of standards on global food trade pattern [20], [21]. That is, several scenarios led to estimates of the effects of aflatoxin regulatory standards in 15 importing (4 developing) countries on exports from 31 (21 developing) countries. It is stated that ifall the countries adopted an international standard of aflatoxin B1 in food [9 µg/ Kg (ppb)] , which would be equivalent to the Codex guidelines of 15 µg/ Kg for total aflatoxins in contrast to all importing countries remaining at the (generally lower) limit of 1998. So doing would lead to an increase in cereal and nut trade among these countries by 61.1 billion USD (51%) [21]. In EU the lowest maximum limit for aflatoxin B1 other than for products for infants is set as 2 µg/ Kg (ppb) for products such as groundnuts (peanuts) tree nuts, dried fruits and its processed products, cereals and products derived from, including processed products (Group 1). The highest maximum limit for aflatoxin B1 is 12 µg/ Kg (ppb) for foodstuffs such as almonds pistachios and apricot kernels. With regard to total aflatoxins, the lowest EU limit other than food for infants is 4 µg/ Kg (ppb) for products of group 1 mentioned earlier. However, the highest limit is set for groundnuts, almonds, pistachios, apricot kernel, hazelnuts and Brazil nuts at 15 µg/ Kg (ppb). That is, the limit varying according to the commodity, but range from 2 – 12 µg/ Kg for B1 and 4 – 15 µg/ Kg for total aflatoxins [22]. Moreover, aflatoxin B1 is the most important of the aflatoxins, considered from the viewpoints of both toxicology and occurrence. It is unlikely that commodities will contain aflatoxins B2, G1 and G2 and not aflatoxin B1. The sum of concentration of aflatoxins B2, G1 and G2 is generally less than the concentration of aflatoxin B1 [23]. The importance of study of aflatoxin contamination in food and feeds stems from the toxicity and carcinogenicity of these chemicals to humans and animals [24]. However, the spores and other viable

propagules of *A. flavus*, *A. parasiticus*, and other fungi can cause three types of disease in humans (allergy, poisoning, and infection) [25]. The *A. flavus* infection in human is uncommon but possible. That is, in Sudan a fatal *A. flavus* infection was reported in a master student at the Faculty of Agriculture, U. of K. who studied it in groundnuts. This infection lead to the death of that student in late 1970s [26]. Another case was a complication of *A. flavus* infection in the sinuses of a female university student. This infection complication was an abnormal mass formation in the oculi that resulted in bulging eye (exophthalamos, proptosis). This case was successfully cured by surgical intervention and strict post operational long term medication [27]. However, this infection might definitely accompanied by toxin secretion from this toxigenic fungus. The disease caused by *A. flavus* is known as aspergillosis and has four patterns (acute invasive, allergic, aspergilloma and chronic necrotizing) [28].

Table 1: Total Aflatoxin in Test Samples from Five Test Firms Using AfalaTest®

Firm No.	Samples / Aflatoxins (µg/ Kg, ppb)				
	1	2	3	4	5
A	0.00	0.00	0.00	0.00	0.00
B	0.00	0.00	0.00	5.87*	0.00
C	0.00	0.00	0.00	0.00	0.00
D	2.45*	0.00	0.00	0.00	0.00
E	0.00	0.00	0.00	0.00	0.00

* Aflatoxin B1.

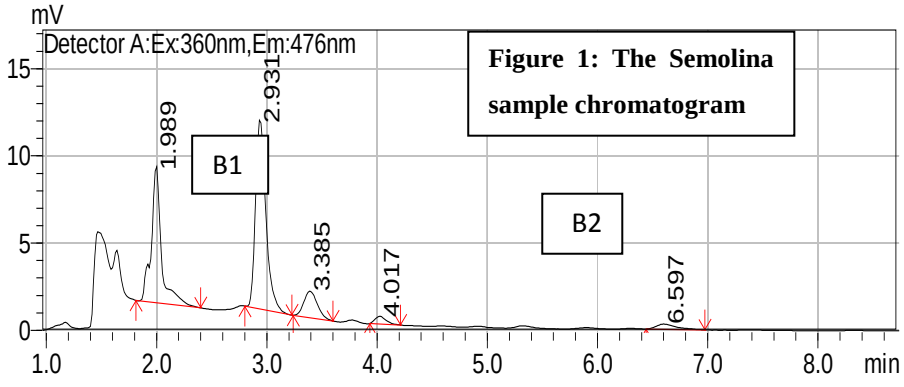

Figure 1: The Semolina sample chromatogram

* B2 is lower than the detection limit.

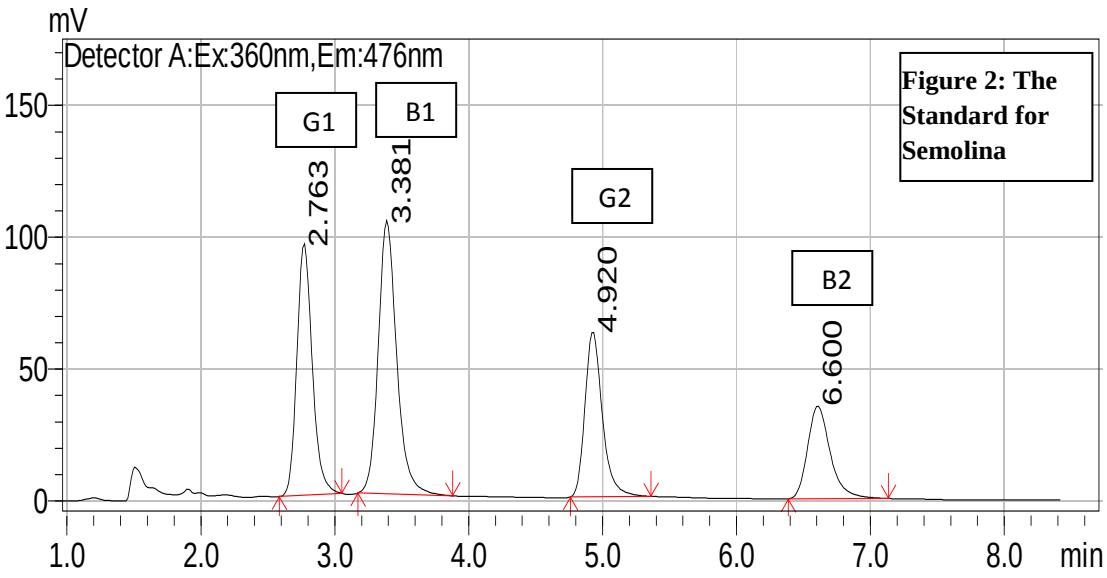

Figure 2: The Standard for Semolina

This panorama of information justifies the importance of studying the aflatoxin contamination of food and feeds including the detection of the standard limits and the specific quantification of these toxins as well that in a route this study runs.

Conclusion

This study quantified the level of aflatoxin contamination in 8% of tested grain and flour samples from the biggest grain stores in Sudan using the AflaTest® HPLC. The recorded levels (2.45 and 5.87 µg/ kg) in only two out of 25 test samples are both less than the standard limit of total aflatoxins set by the Codex Alimentarius (10 µg/ kg). These findings give some alerts to increase the protective measures against aflatoxin formation, and the most dangerous B1, and advocate the safety parameters adopted in the storage systems of the strategic grain sector in Sudan yet more improvement is needed for zero aflatoxin record in these huge stores.

Acknowledgment

I am really grateful to Mr. Hamza G. Omer, Mr. Mohamed G. Omer, the AAA, Australia and the Vicam® family of the Waters Incorporation, USA.

References

[1] Wilkes, M. Biochemistry of grain storage. A Report for Australian Awards in Africa, Postharvest management of maize, rice and legumes course, University of Sydney, Australia, 2012.

[2] Abdullah, N., Nawawi, A., Osman, I. Fungal spoilage of starch – based foods in relation to its water activity (a_w). Journal of Stored Products Research, 36, 2000, 47 – 54.

[3] Vencelli, P., Parker, G. and Mc Neill, S. Aflatoxins in corn, ID – 59. A Publication of College of Agriculture, Food and Environment, University of Kentucky, USA, 2013, P.7.

[4] Genkawaa, T., Uchinob, T., Inouea, A., Tanakab, F., Hamanakab, D. Development of a low-moisture – content storage system for brown rice: Storability at decreased moisture contents. Biosystems Engineering, 99, 2008, 515 – 522.

[5] Wilkes, M. The effect of storage on cereal grain quality. A Report for Australian Awards in Africa, Postharvest management of maize, rice and legumes course, University of Sydney, Australia, 2012.

[6] Shotwell, O. L., Goulden, M. L., Bothast, R. J. and Hesseltine. C. W.Mycotoxins in Hot Spots in Grains. I. Aflatoxin and Zearalenone Occurrence in Stored Corn.Cereal Chem. 52, 1975, 687 - 696.

[7] Miraglia, M., De Santis, B., Minardi, V., Debegnach, F. and Berga, C. The role of sampling in mycotoxins contamination: a historic view. Food Additives and Contaminants, 22 (Suppl. 1), 2005, 31 – 36.

[8] Shepard, G. S., Berthiller, F., Burdaspal, P. A., Crews, C., Janker, M. A., Krska, R. and Whitaker, T. B. Developments in mycotoxins analysis: An update for 2010 – 2011. World mycotoxin Journal, 5 (1), 2012, 3 – 34.

[9] Anderson, M. G., Reiter, E. V. Lindqvist, P., Razzazi – Fazieli, E. and Häggblom, P. Comparison of manual and automatic sampling for monitoring ochratoxin A in barley grain. Food Additives and Contaminants Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 28 (8), 2011, 1066 – 1075.

[10] Berthiller, F., Schumacher, R., Adam, G., and Krksa, R. Formation, determination and significance of masked and other conjugated mycotoxins. Analytical and Bioanalytical Chemistry, 395, 5, 2009, 1243 – 1252.

[11] Berthiller, F., Dall' Asta, C., Schumacher, R., Lemmens, M., Adam, G., and Krska, A. R. Masked mycotoxins: Determination of deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography – tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 53, 9, 2005, 3421 – 3425.

[12] Lancova, K., Hajslova, J., Poustka, J., Krplova, A. Zachariasova, M., Dostalek, P., and Sachambula, L. (2008). Transfer of *Fusarium* mycotoxins and "masked" deoxynivalenol (Deoxynivalenol – 3 – glucoside) from field barley through malt to beer, Food Additives and Contaminants – Part A, Chemistry, Analysis, Control, Exposure and Risk Assessment, 25, 6, 2008, 724 – 744.

[13] Gareis, M., Bauer, J., Thiem, J., Plank, G., Grabley, S., and Gedek, B. Cleavage of zearalenone – glycoside, a "masked" mycotoxin, during digestion in swine. Zentrablatt Fur Veterinandizin. Reihe B. Journal of veterinary medicine. Series B, 37, 3, 1990, 236 – 240.

[14] Berthiller, F., Krksa, R., Doming, K. J., Kneifel, W., Juge, N., Schumacher, R., and Adam, G. Hydrolytic fate of deoxynivalenol – 3- glycoside during digestion. Toxicology letters. 206, 3, 2012, 264 – 267.

[15] Pitt, J. I. Control of aflatoxins by competitive exclusion. Report of CSIRO Food and Nutrition Sciences, North Ryde, N. S. W. 2113, Australia.

[16] Vicam. AfalTest® Instruction Manual 715001733 Rev B. Vicam®, Waters Incorporation, 34 Maple Street, Milford MA 01757, USA, 2011.

[17] Ali, S. A. A personal meeting. The head section of Toxicology, National Chemical Laboratories, Federal Ministry of Health, Khartoum, Sudan. May, 2014.

[18] Ventura, M., Gómez, A., Anaya, I., Díaz, J., Broto, F., Agut, M. and Comellas, L. (2004). Determination of aflatoxins B1, G1, B2 and G2 in medicinal herbs by liquid chromatography – tandem mass spectrometry. Journal of chromatography A, 1048 (1), 2004, 25 – 29.

[19] Codex: Codex General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995), as amended.

[20] Wilson, J. S. and Otsuki, T. Global Trade and Food Safety: Winners and losses in a fragmented system. Report to Development Research Group (DECRG), the World Bank, Washington DC, 2001.

[21] Abbas, H. K. Aflatoxin and Food Safety. Taylor and Francis, Boca Raton, Fl, USA, ISBN 10: 0 8247 2303 1, 2005, pp 83.

[22] EC. Commission Regulation (EC) No. 1881/ 2006 of 19 Dec. 2006, as amended, on Setting Maximum Levels for certain contaminants in Foodstuffs.

[23] Van Egmond, H. P. and Jonker, M. A. Worldwide regulations on aflatoxins. *In*: Abbas, H. K. Aflatoxin and Food Safety. Taylor and Francis, Boca Raton, Fl, USA, ISBN 10: 0 8247 2303 1, 2005, pp. 77 – 93.

[24] ICRISAT. Aflatoxin contamination of groundnut: Proceedings of an International Workshop, 6 – 9, Oct. 1987, ICRISAT Centre, Patancheru, A. P. 502324, India, ISBN 9290661445.

[25] Hill, R. A., Wilson, D. M., Burg, W. R., and Shotwell, O. L. Viable fungi in corn dust. Applied and Environmental Microbiology, 47, 1984, 47 – 87.

[26] Baghdadi, A. M. Personal scientific chat, 1985, Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.

[27] Ahmed, M. E. Personal meeting, 2001, Department of Parasitology, National Health Laboratory, Federal Ministry of Health, Khartoum, Sudan.

[28] Segal, B. H. Aspergillosis. NEJM, 360, 18, 2009, 1870 – 1884.