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Aflatoxin Contamination and Research in China

Huili Zhang^{1,2}, Jianwei He², Bing Li³,
Hui Xiong³, Wenjie Xu³ and Xianjun Meng¹

¹Academy of Food Science Shenyang Agricultural University, Shenyang,

²School of Life Science, Liaoning University, Shenyang,

³Collage of Light Industry, Liaoning University, Shenyang,
China

1. Introduction

Aflatoxins(AF) are highly poisonous secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. They have been found in moldy human food and animal feeds and have been implicated in numerous animal disorders. *A. parasiticus* produces four major aflatoxins: B1, B2, G1 and G2, while AFB1 is the most toxic in the group and the toxicity is in the order of B1 > G1 > B2 > G2. Since the 1960 outbreak of Turkey X disease, when more than 10,000 turkeys died after being fed with aflatoxin contaminated peanut meal, scientists in China have paid more attention to the studies on aflatoxins including its distribution, pollution, health hazards, testing, monitoring, detection technology, managing, microbiology, ecology, toxicology, and policies in controlling aflatoxins. In this review, we present a brief report on the situation of aflatoxin contamination and research progress in China.

2. Distribution of aflatoxin contamination

2.1 The distribution in cereals, oils and foodstuffs

In general, the nationwide aflatoxin contamination was mainly in cereals, oils and foodstuffs. The aflatoxin B1 (AFB1) content detected in vegetable oil products was far higher than in food products. Based on 1,000 investigations of susceptible aflatoxin contamination from nearly 20 provinces between 2002 and 2008, contamination was reported in almost every province. The majority of the samples tested shows the presence of aflatoxins. The overall level of contamination in southern part of China is higher than in the northern region. The most severe province is Guangxi. The main reason is due to the hot and humid southern climate. Climatic condition significant influences the level of aflatoxin contamination. When in serious drought and/or high temperature conditions, or when the soil humidity is below the normal level before harvest, it increase in the number of *A. flavus* spores in the air resulting more fungal infection, and thus high level aflatoxin accumulation. During end processing and packaging, storage of animal-derived food, "cold chain" transfer or pollution of the packing material could also lead to the *A. flavus* infection and aflatoxin contamination (Duan *et al.*, 2009).

Studies on the level of aflatoxin B1 in 486 foodstuff and 146 oil samples collected from 18 cities in 2008 (Zhang, 2008) demonstrated that the levels of aflatoxin contamination were between 0.02 and 54.20 µg/kg in foodstuff and 0.41 and 36.54 µg/kg in oil products respectively. While the detection rate ranged from 0.41% to 2.06%, respectively. A similar study in 2004 reported that the aflatoxin B1 detection rate was as high as 58% in 17 grain samples, which was the most severe incidence in Guangxi province (Wang, 2004). Samples collected from 5 provinces including Sichuan (mainly in Chongqing), Guangdong, Guangxi, Hubei and Zhejiang showed that the aflatoxin B1 detection rates were 70.27% and 24.24% in corn and peanut respectively (Liu, 2006). The aflatoxin B1 detection rates in peanut oil, peanut and corn samples collected from Yunnan province were 100%, 24.32% and 5.26% respectively. The aflatoxin levels in samples were 16.05% above the legally allowed limit, which was similar to the level in peanut samples from Beijing (Wei, 2002; Gao et al., 2007).

2.2 Distribution in dairy products

The investigation of aflatoxin contamination in dairy products indicated that aflatoxin M1 (AFM1), a hydroxylated metabolite of AFB1 secreted in milk, was commonly detectable in most of the dairy samples tested. This phenomenon is correlated well with the distribution of AFB1. From a survey of more than 1,000 samples in 17 provinces between 1991 and 2005, the following reported detection rate ranged from 4.0% to 73.7%.

The scientists of Guangxi Anti-Epidemic and Basic Course Section monitored the AFM1 contamination in 100 samples of milk and dairy products in 15 provinces between 1991 and 1999, the AFM1 contamination levels in milk were from 0.2 µg/kg to 1.9 µg/kg (Tang, 1999a). In 1991, a study using the HPLC on 57 milk samples and 15 milk powder samples from Shanghai, the detection rate of AFM1 were 26.7% and 73.7%, respectively (concentration ranges between 0.025 and 0.95 µg/kg (Zhu, 1991). A similar survey was performed using TLC in 1995 on 59 milk and 53 milk powder samples from Fuzhou, reported that the detection rates were 4.0% and 13.19% respectively (concentration range between 0.06 and 0.20g/kg (Lin, 1995).

The data indicated that the detection ratio of AFM1 correlate with the high content of AFB1 in animal feed. The amount of AFB1 consumption by animals influences the amount of AFM1 secreted in milk in a dose-dependent manner. Again, the highest level was detected in milk and dairy products from Guangxi province.

2.3 Distribution in feed

The investigation of aflatoxin contamination in animal feed demonstrated the wide distribution of aflatoxins. A survey done in more than 1,000 samples in 20 provinces from 2003 to 2008 showed that aflatoxins present in most of the samples, as stated below. This analysis indicated that the general level of serious contamination in southern region is similar to that in northern region. Irradiation has been suggested as a possible means of controlling insects and microbial populations in stored food under moist storage condition (Xiao *et al.*, 2007).

Aflatoxins in feeds has long been a problem in Huanan, Huabei and Huazhong large geographic regions. Detections of AFB1 in 109 samples showed that the aflatoxin detectable rate and the average content were 83.9% and 24.6µg/kg in corn, 100% and 8.27µg/kg in complete feed, 100% and 6.81µg/kg in animal and plant protein, 100% and 13.3µg/kg in

mycoprotein, respectively (Wang *et al.*, 2003). The data showed the relevant ratio, average content and above limit ratio of aflatoxins in feedstuffs were 92.1%, 8.15g/kg and 6.6%, respectively. These values were 100% and 5.95µg/kg in dairy cattle mix feed as studied during 2006 and 2007 (Ao & Chen, 2008).

2.4 Distribution in fermented flavoring

The investigation demonstrated that the safety of fermented flavoring food products such as soy sauce is very optimistic in China. A latest survey of 203 samples of national brand soy sauce samples in 2010 showed that the aflatoxin level is below the maximum allowed level set forth by European Commission (Qi & Che, 2010). This may be contributed by the fact that soybean, raw material of fermentation, is not susceptible to infection of aflatoxin-producing fungi preharvest, even though the growth condition of *Aspergillus oryzae* and *Aspergillus niger*, are similar to that of the aflatoxin-producing fungi.

The maximum amount AFB1 allowed in brewed soy sauce in China was set by law at 5µg/kg. In order to understand the AFB1 contamination of the brewed soy sauce in China, 203 soy sauce samples from different provinces in China were tested for the establishment of emergency response and early warning systems of AFB1 (Sun *et al.*, 2010). The study concluded that the soy sauce is safe for consumption. The average AFB1 content in the brewed soy sauce from the five provinces in China were 0.3560µg/kg, 0.4636µg/kg, 0.5273µg/kg, 0.3143µg/kg and 0.2083µg/kg respectively. All of the tested samples were below the maximum allowed level set forth in China and the EU countries, which is 2µg/kg.

2.5 The distribution in traditional Chinese medicine

Due to a great variety of traditional Chinese medicines and the wide area of planting regions, the traditional Chinese medicinal herbs can be infected with aflatoxin-producing fungus, *A. flavus*, in the process of processing, storage and transportation. Aflatoxin-producing fungus exists in soil and air and Chinese medicinal herbs can be infected by *A. flavus* directly. Studies demonstrated previously that the aflatoxin contamination in Chinese herbal medicine is another issue of concern in preventing aflatoxin contamination in the food.

The investigation of AFB1 in regular Chinese herbal medicine and Chinese traditional patent medicines using the method of ELISA has been reported (Ren & Ma, 1997). It was demonstrated that the presence of AFB1 in traditional Chinese medicinal materials was common. Results suggested that the positive rate and contents of AFB1 were serious enough to alert our concern. Studies in 20 different provinces during 1997~2001 period showed that the AFB1 content in 83%~100% samples was over the limits allowed, with several samples seriously over limits. The indirectly competitive enzyme-linked immunosorbent assay on seven Chinese medicines showed that the aflatoxin content in severe cases reached as high as 200~229 ng/g in Shenqu and 1,056 ng/g in Yueju baohe pellet, respectively (Liu, 2001). Other report showed 85% of samples detected the presence of aflatoxin at a concentration less than 1ng/g (Tang, 1999b).

3. The toxicity of aflatoxin

A series following surveys of nearly 10,000 people from 2006 to 2009 show that the toxicity has a positive correlation with the distribution of AFB1. The total morbidity in the southern region is more serious than in the northern region. Guangxi and Zhejiang

provinces are the high incidence regions, which is significantly higher than the already reported AFB₁ contamination area, Guangdong, Hunan and Singapore. It is widely accepted that aflatoxin contamination in the region is correlated well with the onset of liver cancer in human. Studies showed that AFB₁ causes the p53 gene mutation in human cancer cell. P53 is a tumor suppressor, a transcription factor involved in the regulation of the cell cycle.

3.1 Harm to human

3.1.1 Carcinogenicity

Aflatoxins are the most notorious within mycotoxins. These toxins target liver of human and animal and can lead to hepatic cancer and even death. Among them, the AFB₁ was categorized as No. 1 carcinogen by International Agency for Research on Cancer (IARC) in 1988. A significant negative relationship between the amount of aflatoxin in food with incidence of liver cancer was observed (Gao, 1998).

3.1.1.1 Hazards

To explore the epidemiological feature, as well as the changing rule of the morbidities of malignant diseases, especially, liver cancer, in the population of Fushui county, Guangxi province in the period of 1997~2003 (Huang & Wei, 2006), morbidity data of all malignant diseases in Fushui county, Guangxi province, were collected. Population data were collected as well. The population construction, by ages and sexes, was calculated, referring to the data of overall survey in 1990. They calculated statistically the yearly rates of liver cancer, in order to produce the changing trends comparing the data with history data. Results showed the mean morbidity rate of liver cancer in Fushui county was 52.79/105 (or 50.50/105, if adjusted to the Chinese population, 1964). Liver cancer morbidity rate was the highest in all of the malignant diseases occupying 57.01% of the morbidity rate of overall malignant diseases. Male is more susceptible than female. The ratio of morbidity rate between men and women was 4.93:1. Morbidity rates of liver cancer rose by ages, with the mid-age of 47.58. Morbidity rates of liver cancer in these years remained relatively stable. Comparing with 1970's data, these rates seemed already slightly reduced. Considering that mortality rates (a replacement of cancer morbidity rates) of liver cancer already rise obviously in Guangxi, as well as in the rest area of China. The trend that morbidity of liver cancer in Fusui county reduces a little comparing with historical data. This could be considered as a reflection of the effect of cancer control, which had already been being carried out in Fusui county. The facts that mid-age group of morbidity is decreased and that morbidities in younger age group is significantly reduced are the important evidences of the effect of the field cancer control.

In 2008, polymorphism studies on CYP3A5 genes in 210 patients from high aflatoxin contamination area showed that about 60% of the total individuals are those with high level CYP3A5 expression^[25]. This percentage is far higher than that has been reported in Guangdong, Hunan provinces and Singapore, which are considered low aflatoxin B₁ contamination. Consequently, the contamination of aflatoxin is the main reason of the occurrence of liver cancer in this area (Lu *et al.*, 2008). In the same year, studies on the relationships of the aflatoxin exposure, glutathione transferase gene polymorphism and high risk group with primary hepatocellular carcinoma shown that the exposure of aflartoxin is the main risk factor of the occurrence of liver cancer in this area (Tang *et al.*, 2008).

In 1970~1999, there were 4,215 new liver cancer cases in Zhongshan. Its crude incidence rate, China and world standardized rates were 13.0/105, 12.5/105, 16.8/105, respectively. There is no increasing or decreasing trend for its incidence rates in 1975~1994. However, a declining tendency between 1995 and 1999 was observed. The liver cancer incidence rate during this period in Zhongshan was moderate comparing with the worldwide statistics, but at middle-high level and at low level compared with urban and rural pilot areas in China at the same time period.

The crude and standardized incidences of liver cancer were analyzed by collecting the disease information from the rural area in Ningbo from 2006 to 2008 (Cui *et al.*, 2009). The results show that the crude incidence of hepatoma of the rural residents in Ningbo from 2006 to 2008 is 38.66/105. The age standardized incidence of this disease is 32.14/105. The incidence of hepatoma increased with age. Its incidence in male is 2.77 times of that in female. As to the diagnosis technology, imageology is the most persuasive method to make a definite diagnosis with a ratio of 58.93%. Next effective method is the pathological examination with a ratio of 36.72%. Hepatoma incidence of rural residents in Ningbo is above the average ratio of that in Zhejiang province and China.

3.1.1.2 Pathogenesis

The aflatoxin can result in cancer by a variety of molecular mechanisms.

Aflatoxin exposures can begin in utero and continue through childhood. A mutation in the P53 tumor suppressor gene from AGG to AGT (arginine to serine) transversion at codon 249 (Ser249 mutation) has been reported for hepatocellular carcinoma and matched plasma DNA found in plasma of young children from a region of high aflatoxin exposure (Xu, 2009). This gene mutation in tumor-derived DNA has recently been detected in plasma or serum DNA from adult hepatocellular carcinoma patients. The presence of this mutation before hepatocellular carcinoma onset (e.g., in patients with cirrhosis and patients without clinically diagnosed liver disease) may indicate that the mutation is a marker of chronic exposure to aflatoxin (Kirk *et al.*, 2005). This mutation has been detected in areas with high aflatoxin exposure while it is rare in the low aflatoxin exposure regions (Duan *et al.*, 2005).

A close relationship between the expression of survivin, a newly founded inhibitor of apoptosis protein (IAP), and the abnormality of Wnt signal transduction pathway, was revealed (Ban & Cao, 2005), (Jiao *et al.*, 2007). The HBV is prevalent in high hepatic cancer risk areas, so there is a synergetic effect between the two risk factors. Using population-based case-control study to find the main risk factors of hepatocellular carcinoma (HCC), when people exposed with three main environmental factors (HBsAg, intake of moldy food and drinking raw water), the ORs of hepatic cancer were increased by several times suggesting a conjugated effect between HbsAg and AFB1 albumin adduct.

Concerning the coordinate carcinogenic mechanism between AFB1 and HBV, it is concluded that: i. both of the risk factors can reduce the gene expression level of drug-metabolizing enzyme; ii. Chronic inflammatory reaction increased possibility of p53 mutation induced by AFTB1. iii. Chronic infection of HBV changes AFTB1 to an active form. iv. HBX inhibits the nucleus excision repair of DNA, hindering the repair of AFB1-DNA adduct and similar DNA damage and accelerating the process of carcinomatous change of hepatic cells. Besides, the sensibility of host to AFB1 and fatty degeneration of liver could also come to be carcinogens (Xu, 2009).

3.1.2 Chronic intoxication

3.1.2.1 Correlated event

It is widely reported that the aflatoxins cause many human acute intoxication events. For example, farmers in three families ate mildewed rice (the aflatoxin content reached 225.9 µg/kg) in Taiwan province. This event led to 25 persons poisoned and the deaths of three children, among 39 persons involved. There was an explosion of Toxic Hepatitis caused by aflatoxin in 200 villages, 397 persons got the disease and 106 persons dead (Wu, 2007).

3.1.2.2 Symptoms

After a person ate aflatoxin contaminated food, it may cause fever, abdominal pain, vomiting, more seriously splenohepatomegalia, hepatalgia, skin mucous membrane stained yellow, ascites, edema of lower limbs and dysfunction of liver after 2~3 weeks. The cardiac dilatation, pulmonary edema, coma, spasm may also occur (Xiao & Xing, 2003).

3.2 Harm to animals

The aflatoxin can cause damage to the liver, and the sensitivity of aflatoxin is closely related to animal size, species, gender, age, and nutrition. The aflatoxin can damage to the animal embryo, decrease the liver function, cause a decline in milk and egg production, and decrease animal immunity if infection of micro organism happens repeatedly. During the growth period, animals at young stage are more likely to be infected. The clinical manifestations poisoning include low reproductive capacity, gastrointestinal dysfunction, decline in feed utilization. Moreover, dairy cattle could produce AFM1 and M2.

3.3 Financial loss

According to statistics, the aflatoxins contamination of animal feed in USA led to about 10% financial loss. Besides, the death of livestock results in a severe loss to the agriculture (Zhang, 2008). At the same time, aflatoxins can reduce the production of the food and fiber crops.

4. The biological research

The microbiology research of the aflatoxins in China started fairly late. Reviews on biosynthesis of aflatoxins have not been reported until 2003 and researches on aflatoxin resistance were started only since 2001. Molecular biological methods have been carried out in aflatoxin research during the last two years, such as gene chip (microarray) technology used for gene expression studies.

4.1 Biosynthesis of aflatoxins

4.1.1 Process of synthesis

Based on the improvement in the analysis of the aflatoxins biosynthesis, the aflatoxin biological synthesis process was summarized (Xu & Luo, 2003). In the initial period, with Acetyl-CoA as the original unit and malonyl-CoA as the elongation unit, the reaction is catalyzed by polyketide synthase to form the aflatoxin backbone, polyketone. In general, the specific process of the synthesis scheme of AFB1 and AFG1 is from Acetyl-CoA to caproyl-CoA, norsolorinic acid, averantin, averufin (AVF), versiconal hemiacetal acetate, versiconal, versicolorin B, versicolorin A, versicolorin, *O*-methylsterigmatocystin, and finally to AFB1 and

AFG1; the synthesis of AFB2 and AFG2 is: The front part of the process is the same to AFB1 and AFG1, the difference is versicolorin B changed to dihydro versicolorin, then to dihydro-O-methylsterigmatocystin, to AFB2 and AFG2.

4.1.2 Factors involved in the synthesis

The factors closely related to the synthesis of aflatoxins include the genes, enzymes and the environmental conditions. The genes related to the aflatoxin biosynthesis were analyzed by the technology of gene chip as well as RT-PCR method (Hu & Xu, 2009). Six abnormally expressed genes were detected. The six genes are *aflA*, *aflE*, *aflF*, *aflR*, *aflT* and *aflX*. According to the result, the different expression level of *aflR* has close correlation with the production of aflatoxin.

Some related factors in the synthesis of aflatoxins were studied (Lu *et al.*, 2010). The results show that several dehydrogenases, peroxidases, cyclases, methyltransferases and oxidoreductase have a key role in the biosynthesis of aflatoxins. The activity of those enzymes affected the yield of aflatoxins directly. On the other hand, the most important environmental factors are carbon and nitrogen source, power of hydrogen, temperature, water activity and plant metabolites.

4.2 The resistant research

Studies on resistance to aflatoxigenic fungi through molecular biology in China include: the synthesis of artificial antigens, the aflatoxin resistant microorganism and catabolic enzymes, screening of important resistance genes and molecular markers. Aflatoxins are small molecules, thus the immunization of aflatoxins was achieved through coupling with large proteins. With the m-chloroperbenzoic acid (MCPBA) as oxygenant turning the aflatoxin G1 to 8,9-epoxide, a compound AFG1-BSA was obtained after the epoxide coupling with BSA in a two-phase reaction system (Zhang & Li, 2008). Ultraviolet scanning of the compound showed a significant difference comparing with the scanning result of aflatoxin G1 and a different fluorescence intensity between them, which indicated the coupling of BSA and AFG1. This analytical method promoted the study on the preparation of monoclonal antibody and immunoaffinity column.

The mixture of broad bean and wheat flour during fermentation was used to screen antagonistic bacteria against aflatoxigenic *A. flavus* (Gao & Ding, 2010). A strain L4 with strong antifungal activity against the aflatoxin-producing fungus *A. flavus* was selected using agar medium (BAM). According to its morphological, physiological and biochemical characteristics and 16S rRNA gene sequence homology analysis, L4 was identified as *Bacillus subtilis*. When L4 and *A. flavus* were co-cultured for 15 days, the weight of the mycelium and the production of aflatoxin B1 were both significantly lower than those of *A. flavus* cultured without L4. The accumulation of AFB1 was greatly inhibited, the suppression effective ratio was 93.7%. When L4 culture supernatant was mixed with the spore suspension of *A. flavus* at ratio of 1:1 and then inoculated on corn, the germination and growth of *A. flavus* was completely inhibited.

Using the method of filter paper diffusion, a strain of marine microorganism which exhibited highly inhibitory effect on *Aspergillus flavus* was screened (Kong & Liu, 2010). With the aid of 16S rDNA gene sequence, this marine strain was finally identified as a marine strain of *Bacillus megaterium*. Then, its inhibitory effects on mycelium extending, spore germination and aflatoxin biosynthesis of *A. flavus* were further studied. Quantitative analysis kit for aflatoxins (Beacon) was used to determine the concentration of aflatoxin. The

results showed that this marine strain exhibited good inhibition to the mycelium growth, spore germination and aflatoxin biosynthesis in *A. flavus*. Eighty-seven percent spore (1×10^9 CFU mL⁻¹ *B. megaterium*) and 50.75% aflatoxin (1×10^8 CFU mL⁻¹ *B. megaterium*) were inhibited, compared with control group. The possible mechanism is that some kinds of metabolites secreted by this marine strain can inhibit the mycelium growth and spore germination of *A. flavus*.

Aflatoxin-detoxifizme (ADTZ), being from *Amillariella tabescens*, can effectively decompose aflatoxins. To secretively express ADTZ in *Pichia pastoris* with higher performance, through optimizing the 5' coding region of its cDNA according to the preferred codons of *P. pastoris* (Zuo & Liu, 2007). Two-step DNA synthesis was used to synthesize the cDNA sequence being optimized of ADTZ (OPT-ADTZ). OPT-ADTZ was inserted in the constitutive plasmid pGAPZαA to construct the recombinant plasmid pNOA. pNOA was linearized and then transformed into *P. pastoris* GS115. Then code-optimized ADTZ was constitutively and secretively expressed in *P. pastoris*. In seed of Balsampear Fruit, the antifungal activity of ribosome inactivating proteins (RIPs) were examined (Liu, 2001). In the research aimed at developing a rapid and reliable screening method for selecting *A. flavus* infection resistance in peanut, two DNA markers closely linked with the resistance to *A. flavus* infection were identified using BSA technique. The two specific fragments were about 440bp and 520bp, respectively. They were named as marker E45M53-440 and E44M5-520 (Lei, 2009). The potential usage of the two markers can be in determining or selecting the resistance to the infection by *A. flavus*.

5. Main methods of detection and screening

Monitoring programs have been established to reduce the risk of aflatoxin consumption by human and animals. Analytical testing methods of large numbers of samples of foodstuffs have been developed for rapid detection of Aflatoxins. Current analytical techniques are more accurate in characterization and quantitation of aflatoxins. These include high pressure liquid chromatography (HPLC), Gas chromatography (GC) and serum assay (ELISA), which are much better than the early thin layer chromatography (TLC) technique (Zhang *et al.*, 2008).

5.1 Thin Layer Chromatography (TLC)

Thin Layer Chromatography is a chromatography technique used to separate mixtures, which is performed on a sheet of glass, plastic, or aluminum foil coated with a thin layer of adsorbent material usually silica gel, aluminium oxide, or cellulose. It can be used to monitor the progress of molecule migration to identify compounds present in a given substance and to determine the purity of a substance. TLC can also be used on a small semi-preparative scale to separate mixtures of up to a few hundred milligrams. The mixture is not "spotted" on the TLC plate as dots, but rather applied to the plate as a thin even layer horizontally to and just above the solvent level. For small-scale analysis, TLC can be far more efficient in term of time and cost than chromatography. To analyze the amount of aflatoxin in samples by TLC, the small-scale target can be visible at UV light under 365 nm wavelength. According to the intensity, size and color of the spots on TLC plates, the type and its exact form of the compounds can be determined (Xie, 2007). As the TLC analysis is often affected by many factors, the accuracy of this method is poor. With the improvement of extraction and isolation method as well as the

application of new reagents, the TLC detection becomes a simple and widely used analysis method. It is still used in China today.

5.2 High Pressure Liquid Chromatography (HPLC)

Using the liquid chromatography (HPLC) method with immuno-affinity column cleanup through post-column derivatization system, aflatoxins can be adsorbed in the immunaffinity column and eluted with organic solvent. The HPLC method with fluorescence detector using post-column derivatization system is a commonly used method in different countries (Wang, 2004). This method is more sensitive and accurate. Furthermore, this method is one of the best method for determining aflatoxin in traditional Chinese medicines (Ma, 2007).

5.3 Micro-column method

Employing the micro-column method to analyze aflatoxin is first to build up the micro-column chromatography tube using sample-extracted solvent and then the aflatoxin would be adsorbed by the florisil adsorbent as the alumina absorb the foreign matter. Under 365 nm UV light, the amount of aflatoxin can be calculated by the intensity of the blue-violet light reflected from the compound. This method is accurate, simple, rapid and reproducible. However, the micro-column is considered a qualitative method (Xie, 2007).

5.4 Enzyme-linked Immunosorbent assay (ELISA)

ELISA is widely used in food and feed industries to determine the content of aflatoxin in food products. Though the ELISA is accurate stable and reproducible, the analysis sometimes shows false positive, or false negative due to enzyme instability and variations of enzymes reaction conditions. So the application of this method in analysis of aflatoxin remains to be improved in future (Ma, 2007).

5.5 Immunochromatography

Immunochromatography is a kind of immunoassay technique developed in recent years. It is simple, rapid and is suitable for prescreening a large number of samples and for analyzing on the spot (Ma, 2007).

6. Prophylactico-therapeutic measures

Efforts to minimize adverse effects of aflatoxins include monitoring, managing and controlling their levels in agricultural products from farm to market and to table. While an association between aflatoxin contamination and inadequate storage conditions has long been recognized, studies have been focused on developing commercial crop cultivars that are resistant to *Aspergillus flavus*, such as peanut varieties Guihua 22 and Yueyou 58. Meantime, selecting the rational planting techniques and harvesting method, reasonable storage conditions and inhibitor are equally important.

6.1 Control measures in oils and foods

6.1.1 Selecting the crop cultivar with high level of resistance to aflatoxins

To date, many countries paid much attention to researches on the development of this method. In China, the new peanut cultivars such as Guihua 22 and Yueyou 58 have been cultivated (Wang, 2004). Since the Vitamin E is an essential factor in the synthesis of

aflatoxins, it is important to select a cultivar with low Vitamin E content in seed coat to reduce aflatoxin contamination.

6.1.2 Using reasonable planting techniques and harvesting methods

It is very important to use suitable planting technique and harvesting method. The unsuitable handling process will cause damage to kernel, which will result in fungal infection and aflatoxin contamination. So during the harvest and storage, any measures that can reduce physical damage to the kernel of crops including insect pest and rats will surely reduce fungal growth and aflatoxin contamination (Wu, 2007).

6.1.3 Using reasonable storage condition

The storage condition is also an important determinant for reducing aflatoxin contamination. The AFB1 content of dry hot peppers stored under different storage conditions was analyzed to provide the theoretical basis for improving quality of dry hot pepper during storage. The results showed that the storage conditions of low temperature, low moisture content, low relative humidity and sealed package could significantly reduce the occurrence and accumulation of AFB1 in dry hot pepper.

6.1.4 Reasonable adoption of antiseptic

Utilizing antiseptic agents is effective in preventing aflatoxin contamination. The most commonly used antiseptics are sodium benzoate, sorbitol, propionic acid and propionates. The antiseptic compounds that contain propionic acid, propionates and sorbitol are in high demand. Studies showed that the removing rate of AFG B1 was more than 90% when using ozone to treat AFG B1 in contaminated crops (Luo *et al.*, 2003).

6.2 Control measures in feed

Except low temperature, low moisture, control of oxygen and using antiseptics, adequate dilution may also prevent the aflatoxin contamination in feed (Wang & Zhang, 2006). However, this method is only applied to the least aflatoxin contaminated situation. The specific dilution methods are to analyze the exact content of aflatoxins and mix those feed for which their toxin content is in the borderline with the unmolded feed materials according to the normal feeding amounts. However, if the diluted feed hasn't been used up soon, it will extend aflatoxin contaminations. The regulating strategy include: based on the fact, adding methionine and electrolyte to improve the hepatic function and increase the natural concentration, especially the level of non-contaminated proteins.

6.3 Control measures in fermented condiment

Control of aflatoxin in fermented condiment should start from the raw materials. Controlling the key factors such as water activity in the soy bean and the humidity in environment is important. The best parameter should be controlled to keep the water content in soy bean below 13% and the humidity no less than 65% in depositories (Wang & Z., 2009). Combined with the application of antiseptic and ozone treatment the quality of the finished products and semi-finished products can be enhanced.

6.4 Control measure in chinese medicines

Similar to fermented condiment, the control of aflatoxin in Chinese medicine should also start from the resources. Thus, control measures of the herbal medicine can be taken to

enhance their resistance based on a developed management practice (Tang, 1999b). In recent years, a new technique of utilizing antagonistic microorganisms and the change from wild fermentation to pure fermentation are becoming effective measures to reduce aflatoxin contamination. Similar to crops, the control methods in storage also include the temperature, humidity, and oxygen etc.

7. Methods of degradation and removing

The practical methods applied for reducing aflatoxin production are: sorting, processing, and the sun light (ultraviolet) sterilization. These traditional methods have been used since 1995 in China. Significant emphasis has been placed on detoxification of contaminated lots by irradiation, ammonia fumigation, chemical method, oxidants, microbial and enzymatic methods commonly used in treating corn and peanuts.

7.1 Adsorption techniques

Adsorption is the most common method to reduce aflatoxin contamination. For example, the harmful damaging effect of aflatoxins to animals can be reduced by adding several nutrition unactive adsorbents to the feed. Additionally, the adsorbents also can remove a portion of the aflatoxins (Xu *et al.*, 2001). Among which the activated charcoal, mannan oligosaccharide (MOS), aluminosilicate, hydrated aluminosilicate and bentonite have been best studied. However, the positive effect has been observed only in the laboratory. Commercial utilization of the absorption method is rarely used in practical production in China.

With nanomaterial silicate adsorbent added to the feed contaminated by aflatoxins, it can significantly reduce the residual toxin in chicken muscle and liver. This is promising for producing safe animal products that meets international standards (Feng, 2004). The new adsorbent can also effectively reduce its harmful effect on growth, visceral function and the immune system of boilers. In the study on the adsorption of Silicate structure adsorbent NSP in feed of pigs, it was discovered that the absorption function in three forms: adsorption inside layers, adsorption between layers, and adsorption at the edges (Qi, 2002). Absorption of several organic adsorbents (KGM, Detoxification substance, Sorbent C) detoxification of AFB₁ in animal was studied (Yu, 2007). *In vitro* experiment to study the absorption characteristics through different adsorption, different content, different pH, and different temperature indicated that in high temperature, absorption capabilities of the three sorbents are worse than that in low temperature. Absorption capability of KGM is very weak in high temperature, while detoxification substance and sorbent C are obviously better. The three sorbents adsorb better in alkaline than in acidic conditions. But in acidic pH, sorbent C is worse than the other two.

Qingdao Agricultural University tested glucomannan to adsorb aflatoxins, the EGM at the concentration of 78.54%, 83.71% and 0.11% showed the best AFB₁ adsorption ability, when the concentration of glucomannan is 0.11% (Yu, 2007).

7.2 Aflatoxins detoxification by ammonia gas

Aflatoxin detoxification in peanut and peanut meal by ammonia gas was tested (Liang, 2009). Single factor test showed that ammonia temperature, time and water content of samples greatly affected AFB₁ degradation. The optimal conditions for best result are 10% ammonia by volume, 24% peanuts meal moisture, which gave 100% AFB₁ degradation. There is no detectable AFB₁ after ammonia fumigation (Liang, 2009).

7.3 Alkali refining

Studies by Liuzhou Health and Epidemic Prevention Station and Food Bureau suggested that one part contaminated feed immersed in two parts NaOH should be boiled for 1~2h before feeding to animals. In addition, use lime cream, pure potash and kali to soak aflatoxin contaminated corns for 2~3h, followed by washing in clean water, and drying. The detoxication efficiency can reach 60%~90% (Fan, 2003).

7.4 Oxidants

The treatment with 5% sodium hypochlorite for several seconds could reduce aflatoxin by 98%~100% (Zhang *et al.*, 2004). After analyzing the difference among different time and the products of different places, the ClO₂ was effective in detoxicating aflatoxin when the aflatoxin contaminated corns were infused in the 250ug/mL ClO₂ for 30~60 min (Zhang & Zhu, 2001). The treatment with 2% sodium bisulfite for 3 days showed best effect on aflatoxin detoxication (Feng, 2002).

7.5 Micro-organisms

The aflatoxin degradation ability of some food micro-organisms such as the lactic acid bacteria and yeasts was investigated (Zhu & Lin, 2001), (Li *et al.*, 2003). The concentration of aflatoxin, the quantity of fungus and the temperature have a combined effect on the toxin binding ability by lactic acid bacteria. In yeasts, in exponential phase, it showed highest toxin binding ability and the higher concentration of aflatoxin, the higher the binding ability. The enzymatic detoxification of aflatoxin is an effective and safe method, highly selective, no harmful effect on nutrition value and no adverse effect to the treated products (Gong *et al.*, 2004). A new technology on aflatoxin detoxification was developed in recent year. Thoroughly enzymatical hydrolyzation of the peanut meal to achieve full ionization of slightly dissolved aflatoxins from hydrophobic amino acid residues. Then retain the greater part of aflatoxin through successive filtration, thereby make markedly reduction of aflatoxin content (Xu & Luo, 2003).

8. The laws and regulations in controlling aflatoxins in China

Due to the risk of aflatoxin contamination of foods and feed on human health and livestock productivity, the Chinese Government has imposed laws and regulations limiting total amount of aflatoxins allowed in foodstuffs and feedstuffs. This has minimized potential exposure to aflatoxins. The maximum level of aflatoxins allowed in many commodities has been established. "Food Hygiene Law of the People's Republic of China" specifically prevents the sale of aflatoxin contaminated commodities and has set limits in food no more than 20 ppb total aflatoxins, and 10 ppb in rice and 0.5 ppb AFM1 in milk, butter and fresh pork. There is a zero tolerance in infant formula that no trace amount of aflatoxins shall be detected.

8.1 Related laws about aflatoxins

At present, Chinese laws about aflatoxin contamination are greatly improved. The Ministry of Health of the People's Republic of China has established a number of hygiene control measures to prevent aflatoxin contamination.

Food hygiene law of the People's Republic of China warns that the food, which is mould or mixed with foreign matters or those with abnormal flavour properties, may be harmful to

human health. "Food Hygiene Control Regulations Article IV" stated clearly that rural and state-owned farms should be organized and guided to harvest in time, threshing, dry, removing impurities, to prevent food mildew pollution during harvesting process. Article VI points out that we should actively carry out the "no worms, no mildew, no rat, and no sparrow" activities. ARTICLE II and III of Prevention Aflatoxin Contamination on Food Hygiene Regulations make clear that we should prevent food mildew and deterioration to achieve the objectives of mould proof and poison removal. Article IV provides that when using grain and oil whose aflatoxin content is higher than allowed level, effective measures must be taken to remove the toxins through technical procedures. The products can only be consumed when the product meets the food safety criterion. Article VI requires that, to ensure infant food safety, a zero tolerance policy should be adopted and food sector should provide non-aflatoxin detectable grain, as materials of infant milk replacer. For aflatoxin monitoring and management, Chinese Health and Quarantine law also established relevant regulations.

8.2 The organizations involved in aflatoxin control supervision

Not one or two departments can accomplish aflatoxin control supervision in the process of strengthening food safety supervision system. Team work may play an important and positive role. Management of aflatoxin control mainly involves the following departments.

8.2.1 Hygiene management department

Due to the problem with aflatoxin contamination during food processing, transportation and marketing process, especially peanuts. The hygiene administrative departments are required to perform some relative control measures on preventing aflatoxin contamination, e.g , Food hygiene law of the People's Republic of China.

8.2.2 Health and quarantine departments

Aflatoxin contamination of food is difficult to prevent, therefore, the aspect of food quarantine is particularly important. China has made specific provisions on the highest aflatoxin tolerance amount in all kinds of food. The health and quarantine departments must adopt the advanced science and technology in aflatoxin testing, strictly implement supervision, to reduce aflatoxin hazard to human health.

8.2.3 Disease control department

Because aflatoxin is extremely poisonous substances, it has an aneretic role on human and animal's liver tissue, accompanied with stem cell degeneration and necrosis, eventually result in serious organ damage or even death. Aflatoxins not only damage liver organ in animals, but also affect embryo development in animal. Due to immuno-suppression and recurrent infections aflatoxin contamination in animal feed will reduce milk and eggs production. Experimental results show that aflatoxin toxicities are different depending on animal species, age, and gender. In general, the younger the animals the higher the sensitivity to aflatoxins. Aflatoxins can also pass through food chain to human body through consumption and accumulation in animals. Disease control department should create a healthy environment, maintaining the social stability and national security, improve people's health through the prevention and control of diseases resulted from aflatoxin contamination. Under the leadership of the ministry of health, technological management and technical service will be enhanced.

8.3 Aflatoxin quarantine requirements

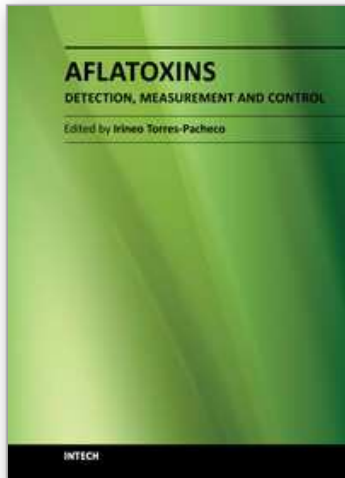
Chinese government has strict regulations on the maximum amount aflatoxin allowed in different foodstuffs. In corn, peanuts, peanut oil, nuts and dried fruit (walnut, almond) the maximum amount allowed is 20µg/kg(AflatoxinB1);. While in rice and oils (sesame oil, rapeseed oil, soybean oil, sunflower oil, oil, tea oil, sesame oil flax, corn germ oil, rice bran oil, cottonseed oil) is 10µg/kg (aflatoxin B1). In milk, milk products and butter (disinfection, fresh raw milk, whole milk powder, and evaporated milk, sweet condensed milk, butter) is 0.5 µg/kg (Aflatoxin M1). No aflatoxins shall be detected in any infant formula.

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Aflatoxins - Detection, Measurement and Control

Edited by Dr Irineo Torres-Pacheco

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This book is divided into three sections. The section called Aflatoxin Contamination discusses the importance that this subject has for a country like the case of China and mentions examples that illustrate the ubiquity of aflatoxins in various commodities. The section Measurement and Analysis, describes the concept of measurement and analysis of aflatoxins from a historical perspective, the legal, and the state of the art in methodologies and techniques. Finally the section entitled Approaches for Prevention and Control of Aflatoxins on Crops and on Different Foods, describes actions to prevent and mitigate the genotoxic effect of one of the most conspicuous aflatoxins, AFB1. In turn, it points out interventions to reduce identified aflatoxin-induced illness at agricultural, dietary and strategies that can control aflatoxin. Besides the preventive management, several approaches have been employed, including physical, chemical biological treatments and solvent extraction to detoxify AF in contaminated feeds and feedstuffs.

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Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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