

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.

For more information visit www.intechopen.com



Aflatoxin Contamination Distribution Among Grains and Nuts

Eduardo Micotti da Gloria
University of Sao Paulo – ESALQ,
Brazil

1. Introduction

Grains (cereals and oilseeds) and nuts in general are subject to mold attack, in preharvest and postharvest. Among molds that can attack these foods *A. flavus*, and *A. parasiticus* are important because they can produce aflatoxins that are considered a potent natural toxin (Wild & Gong, 2010). Aflatoxin can be produced mainly by different *Aspergillus* species, but *Emiricella* and *Petromyces* have been reported as aflatoxin producers (Frisvad et al., 2005).

Aflatoxin contamination has been reported for grains as corn, soya, wheat, rice, and cottonseed, and nuts such as peanuts, almonds, Brazil nuts, hazelnuts, walnuts, cashew nuts, pecans, and pistachio nuts (Fuller et al., 1977; Ayres, 1977; Moss, 2002; CAST, 2003; Gürses, 2006). Despite aflatoxin contamination having been observed in several foodstuffs, the contamination of maize, peanuts, and oilseeds can be considered, in terms of diet exposure, the most important worldwide (Benford et al. (2010).

Based on deleterious problems that aflatoxin can cause to human and animal health, some countries established a maximum concentration for aflatoxins in specific products. According to published data (Van Egmond, 2007), until 2003 one hundred countries had established legal limits for mycotoxins, and most of them regulated the aflatoxins presence in food and feeds.

Several biotic and abiotic factors can determine fungal infection and growth, as well as aflatoxin production in preharvest. Temperature, water availability, plant nutrition, infestation of weeds, birds, and insects, plant density, crop rotation, drought stress, presence of antifungal compounds, fungal load, microbial competition, substrate composition, and mold strain capacity to produce aflatoxin are some important factors. The incidence of these factors is different in preharvest among plants and production areas of the same farm, among different farms of the same region and among different producer regions. Even among grains of the same ear or peanuts of the same pod the differences can occur. In postharvest, factors such as temperature, availability of water, oxygen, and carbon dioxide, insect and rodents infestation, incidence of broken grains or nuts, the cleaning of the product, toxigenic fungal load, microbial competition, antifungal compound presence, and substrate composition are important too. Transport, waiting time for drying, drying system (temperature and drying rate), and storage conditions can affect these factors during the postharvest period (Dorner, 2008; Diener et al., 1987; Campbell et al., 2006; Molyneux et al., 2007).

As a result of variable conditions that can occur during pre and postharvest, the aflatoxin contamination level among grains and nuts within the same lot can have an extremely

uneven distribution. The uneven distribution of aflatoxin contamination was observed in different foodstuffs, such as peanuts, maize, almonds, Brazil nuts, and pistachios (Cucullu et al., 1966; Whitaker et al., 1994; Shotwell et al., 1974; Schatzki & Pan, 1996; Steiner et al., 1992; Shade et al. 1975; Ozay et al., 2007). In a contaminated lot, just a few grains and nut kernels can have quite high concentration levels of aflatoxin, and most of them do not have detectable contamination. Table 1 shows some high individual concentrations detected in a peanut, a maize grain, a Brazil nut, in a pistachio, and a cottonseed. The high concentration observed in an individual grain or kernel can result, for example in maize, in a contamination level of 136 µg/kg, when just one grain is contaminated, considering 0.34 g as the average weight of maize grain, and the high concentration showed in the table 1.

The not uniform distribution of contamination within a lot represents a great challenge to measure the true contamination level of the lot. If several samples are collected from the same lot of a commodity, completely different contamination results can be obtained, as shown in table 2. Several theoretical distribution models have been investigated as possible models to describe the observed distribution of aflatoxin test results. Among them, are the negative binomial (Whitaker et al., 1972; Whitaker & Wiser, 1969; Knutti & Schlatter, 1978; Knutti & Schlatter, 1982), compound gamma (Knutti & Schlatter, 1978; Knutti & Schlatter, 1982; Giesbrecht & Whitaker, 1998), log normal (Giesbrecht & Whitaker, 1998; Brown, 1984), truncated normal (Giesbrecht & Whitaker, 1998), Weibull (Weibel, 1977), 3-parameter Weibull (Sharkey et al., 1994; Schatzki, 1995), exponential, chi-square (Tiemstra, 1969), logistic, and Neiman-A (Whitaker et al., 1972). Additionally, evaluations of several sampling plans to detect aflatoxin contamination have been done, and they have shown, with some differences due to plan characteristics and product to be sampled, that results obtained by sampling plans always involve a certain degree of uncertainty (Whitaker et al., 2005b).

Product	Aflatoxin b1 concentration reported (µg/kg)	Reference
Brazil nuts	4,000	Steiner et al. (1992)
Brazil nuts	25,000	Stoloff et al. (1969)
Pistachio nuts	1,400,000	Steiner et al. (1992)
Peanuts	1,100,00	Cucullu et al. (1966)
Maize	400,000	Shotwell et al. (1974)
Cottonseed	5,750,000	Cucullu et al. (1977)

Table 1. Concentration reported for individual grain or nut

Lot number	Aflatoxin analysis (µg/Kg)										Average
1	0	0	0	0	8	8	15	16	16	125	19
2	0	0	0	0	0	0	0	8	22	198	22
3	0	0	0	0	0	0	0	9	12	285	31
4	5	12	56	66	70	92	98	132	141	164	84
5	18	50	53	72	82	108	112	127	182	191	100
6	29	37	41	71	95	117	168	174	183	197	111

Source: Dickens and Whitaker (1986)

Table 2. Example of variation that can be observed among sample results when a peanut lot is sampled

Despite uneven contamination representing a problem for the task of sampling, it consists in an opportunity to segregate aflatoxin contaminated grains and nuts from an entire lot. As contamination is concentrated in few grains or nuts the removal of those material can to reduce the aflatoxin levels.

The fungal growth in grains and nuts is normally related to some changes in their biochemical and sometimes in the visual characteristics (Pomeranz, 1992; Wacowicz, 1991). Discoloration or staining of skin or kernel material, appearance of fluorescent material, changes in the standard of reflectance and transmittance spectroscopy, density and size changes in relation to sound grains and nuts are some characteristics that have been observed as consequence of fungal growth (Kumar & Agarwal, 1997; Pomeranz, 1992).

Some technologies able to detect and remove grains and nuts with the previously mentioned differences in their characteristics have been studied and used to improve the overall quality of commodities, but their efficiency to be used as a way to reach a reduction of aflatoxin levels in specific commodities must be evaluated. Table 3 shows some technologies which have been studied and used to segregate aflatoxin contamination in lots of commodities.

Technology	Product
Electronic color sorting	Grains and nuts
Hand picking	Nuts
Blanching and electronic color sorting	Peanuts
Gravimetric table	Grains and nuts
Size separation	Grains and nuts
Flotation	Maize and peanuts

Table 3. Examples of technologies studied to improve the overall quality of commodities or to reduce aflatoxin contamination levels of an entire lot

2. Segregation by appearance features

Fungal growth can cause chemical changes in grains or nuts, which can result in some modifications in color or form. The modifications are not always visible to the naked eye, some of them can be visible just with the aid of specific techniques or equipment. Color changes in grains or nuts can appear as a result of biochemical reactions or due to the fungal mycelium itself. According to Robin et al. (1995), hydrolysis of the macromolecules, e.g., proteins, lipids, and polysaccharides, occurs during mold infection, resulting in the release of free amino acids, free fatty acids, and simple sugars. These breakdown products contribute to color development in, e.g., peanut kernels during roasting or blanching before electronic color sorting.

The detection of fungal changes in grains and nuts makes it possible to know where fungal growth, and probable mycotoxin production, has occurred. As the presence of a fungus does not assure mycotoxin presence (Gloria et al., 2006), some researchers have tried to show correlations between changes in grains or nuts and their mycotoxin concentration. The correlation between poorly graded categories of grains and nuts and aflatoxin concentration has been shown for peanuts, maize, and almonds (Whitaker et al., 1998; Johansson et al., 2006; Whitaker et al., 2010).

The optical detection of faulty grains, nuts, kernel of nuts (blemished, discolored, and misshapen), and gross contaminants (glass, stones, insects, rotten product, extraneous

vegetable material, etc.) has been carried out by visual color sorting (hand picking) or by an electronic color sorting (automatic sorting). Sorting of food products using the human eye and hand is still widely practiced where labour rates remain low. However, where the cost of labour has increased, automated techniques have been introduced (Bee and Honeywood, 2002).

There are several possible characteristics in the appearance which have been studied as indicators of aflatoxin presence. The BGYF (Bright Greenish-Yellow Fluorescence) was studied as an aflatoxin contamination indicator to maize (Shotwell & Hesseltine, 1981), pecans (Tyson and Clark, 1974), pistachio nuts (Dickens and Welty, 1975), dried figs (Steiner et al., 1988), and Brazil nuts (Steiner et al., 1992), as shown in Figure 1. The BGYF is produced by the oxidative action of heat-labile enzymes (peroxidases) in living plant tissue on kojic acid, which is produced by *A. flavus*. The method is not a definitive indicator of aflatoxin because it can produce false positive or negative results. False negative occurs when the aflatoxin contaminated maize grain does not present the fluorescent compound because peroxidase or kojic acid were not present to produce it. False positive occurs when contaminated maize sometimes does not exhibit BGYF, while kernels infected with *A. flavus* strains that produce kojic acid but do not produce aflatoxin exhibit BGYF, and thus are aflatoxin “false positives” when a maize grain is examined with a black light (Wilson, 1989; Wiclow, 1999). Hadavi (2005) studied the application of BGYF to segregate contaminated pistachio nuts, and concluded that the BGYF can be used to remove nuts with high aflatoxin level. Nowadays, BGYF is not currently used as a technique of decontamination of aflatoxin contaminated maize, it has been used as a technique for analyzing samples to detect aflatoxin contamination.

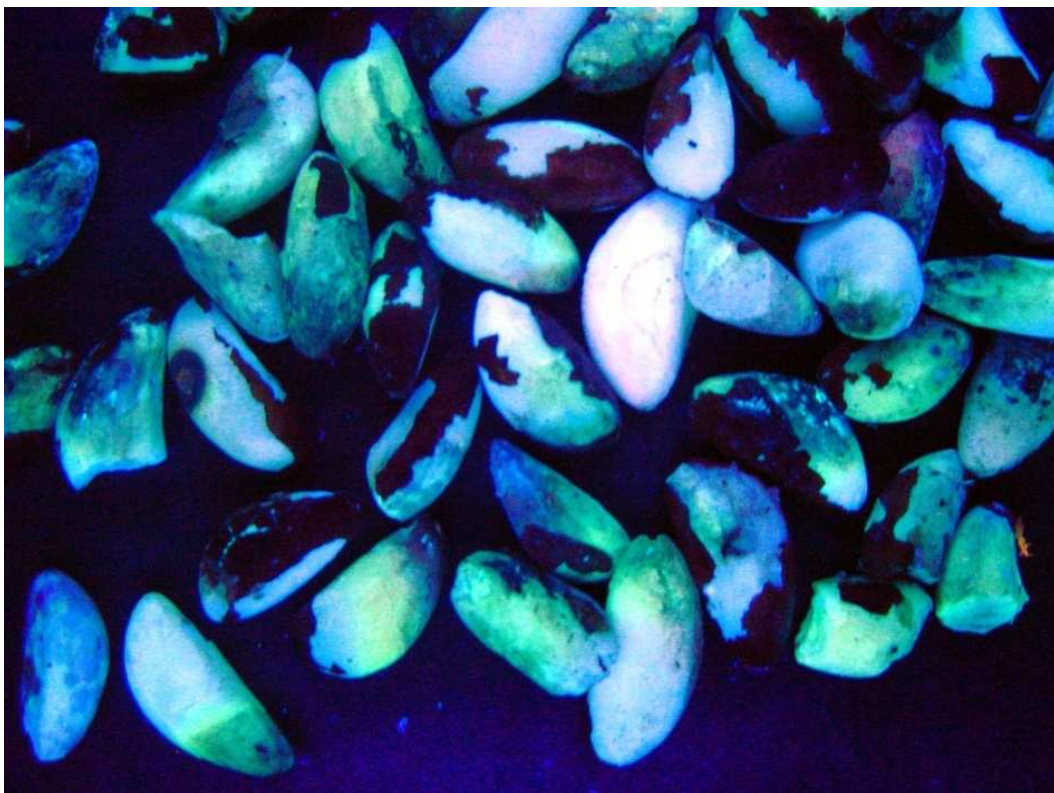


Fig. 1. Brazil nut kernels with Blue Greenish-Yellow Fluorescence (BGYF)

Other types of fluorescence have been studied as a way to indicate contaminated peanuts, almonds, and maize (Pelletier & Reizner, 1992; Shade and King, 1984; Yao et al., 2010). A device capable of measuring fluorescence intensities from peanut surfaces and physically rejected peanuts having undesired fluorescence properties was described (Pelletier et al., 1991), however a comparison of the efficiency between it and the color sorting process in peanuts lots showed that it was not effective as an aflatoxin decontamination technique (Pelletier & Reizner, 1992). Farsaie et al. (1981) developed an automatic sorter to remove fluorescent in-shell pistachio nuts, and an aflatoxin reduction by ca. 50% was reported. Steiner et al (1992) showed that fluorescence (yellow fluorescence) was a good indicator for aflatoxin contamination in kernels of Brazil nuts, but it was not good for in-shell pistachio nuts or kernels of pistachio nuts. For Brazil nuts, the hand picking segregation based on segregation of kernels with fluorescence has been used in Bolivia as an aflatoxin decontamination technique. Yao et al. (2010) reported good correlation between single kernel fluorescence hyperspectral data and aflatoxin concentration in maize.

Despite the fluorescent characteristic of grains and nuts being a possibility to segregate contaminated material, nowadays other color characteristics are used more often as an aflatoxin reduction technology. Color changes can be detected by the naked human eye or by optical systems using different technologies (Bee & Honeywood, 2002). Color sorting by the human eye and hand picking has been used as a feasible process to improve overall quality of nuts, mainly in some world regions where the cost of labour is sufficiently low to justify the economic feasibility of the process. For grains such as cereals, even in regions where the cost of labour is low, hand picking is not a feasible process. In spite of its higher cost in developed countries, hand picking is still used in certain cases to achieve a better removal of contaminated material and aflatoxin reduction, as happens to peanuts in the USA (Kabak et al., 2006).

The efficiency of color sorting to improve overall quality and also to reduce aflatoxin contamination depends on the product and the characteristics of the hand picking process or electronic sorter used. Electronic color sorting segregates grains or nuts with color off-standard in relation to a defined standard for sound grains and nuts which present low probability of aflatoxin contamination (Bee & Honeywood, 2002). Color sorting can be used alone or together with other processes such as blanching used for peanuts. Some reports on the performance of the electronic color sorting to reduce aflatoxin contamination have been published. Dickens and Whitaker (1975) showed that hand picking was more efficient to segregate aflatoxin contamination than electronic color sorting, as the latter also showed variable performance in aflatoxin reduction depending on the lot processed, however a great improvement in the optical technology occurred in the last thirty-five years, therefore nowadays it is correct to believe that color sorters have a better performance than before. Shade et al. (1975) also reported a better efficiency of the hand picking than the electronic color sorting to segregate aflatoxin contamination in almonds. Escher (1974) observed that color sorting was not successful in pecans because inherent intense fluorescence in the kernels. They investigated electronic color sorting and hand picking finish almonds products and they found contamination just in the electronic finish product. However, a great improvement in the optical technology occurred in the last thirty-five years, therefore nowadays is correct to believe that color sorters have a better performance than that time. Whitaker (1997) reported an evaluation of the performance of blanching and electronic color sorting process applied to 8911 contaminated peanut lots during the years of 1990 to 1994, as shown in table 4. The

average reduction of aflatoxin contamination reported was of 89.9% and weight loss of 16.8%. Pearson (1996) reported a machine vision system to automatically segregate stained pistachio nuts which presented hulls with abnormal coloration, which is an indication of nuts with early splitting hulls. The early splitting pistachio nuts present higher probability to be contaminated with aflatoxin than the not stained nuts or nuts with closed hulls (Sommer et al., 1986). Two years later, Pearson & Shatzki (1998) reported an evaluation of this system and concluded that the sorter could be applied in the product recovery, and in the preparation of the product for very stringent markets. Visual sorting with hand picking based on color characteristics has been used for improvement of the overall quality of nuts, e.g. peanuts and shelled Brazil nuts in some processing plants in Brazil (Figures 2 and 3). Galvez et al. (2002) proposed a method to reduce aflatoxin in raw peanuts based on roasting, manual de-skimming and human sorting. The method was able to reduced aflatoxin of high and low contaminated samples. Campbell et al. (2003) observed that for walnuts the main commercial sorting used in the USA was based in color sorting to separate light colored shells (high value) from darker shells, and darker shells contained some shriveled and darkened kernels but until that time there was not information about the correlation of those types and aflatoxin content. De Mello & Scussel (2009) evaluated different types of sorting processes and concluded that color sorting for in-shell Brazil nuts did not show a safe segregation of contaminated nuts.

Crop Year	Lots processed	Aflatoxin contamination ($\mu\text{g}/\text{Kg}$)		Reduction (%)	Weight loss
		Before	After		
1990	5479	56.3	3.6	90.7	16.8
1991	669	36.6	2.5	92.0	14.7
1992	311	33.0	2.5	90.4	13.9
1993	1861	35.8	3.6	88.0	18.9
1994	591	31.0	3.4	86.6	14.1
Average/Total	8911	48.1	3.5	89.9	16.8

Source: Whitaker (1997)

Table 4. Aflatoxin reduction in contaminated peanut lots after blanching and electronic color sorting

Color sorting technology has shown several innovations over the last years which have improved the efficiency to remove poor quality grains, nuts and extraneous material (Bee & Honeywood, 2002). According to Wicklow & Pearson (2006), sorters used in the past had limited capacity to separate molded products because their optical system was based on mono-chromatic red-filters. However, the near-infrared is nowadays a feasible technology to be used in sorters, thus bi-chromatic color sorters have had their capability of detection extended beyond visible light, which made it possible to detect color and bio-chemical changes due to fungal growth. Sorters have used near-infrared transmittance (NIRT) and near-infrared reflectance (NIRR) spectroscopy to evaluate internal quality in many whole nuts.



Fig. 2. Hand picking of peanuts based on color and other characteristics of kernel



Fig. 3. Sorting of Brazil nuts kernels based on color and other characteristics of during hand shelling step

Some color sorters using those innovations were checked to evaluate the performance of aflatoxin segregation. Hirano et al. (1998) evaluated a method of transmittance near infra red to detected mouldy peanuts and could distinguished mouldy from sound nuts by transmittance ration of 700 nm to 1100 nm. According to authors the triglycerides hydrolysis caused by fungal growth was responsible for spectral differences. Pearson et al. (2001) evaluated transmittance spectra (500 to 950 nm) and reflectance spectra (550 to 1700 nm) to distinguish aflatoxin contamination in a single whole maize grain. More than 95% of maize grains were correctly classified as containing either high (>100 ppb) or low (< 10 ppb) levels of aflatoxin. Classification accuracy for kernels between 10 and 100 ppb was only about 25%, but according to researches these grains do not usually affect sample concentrations and are not as important. Pearson et al. (2004) evaluated a commercial sorter based on that technology and observed a reduction of 81% and 85% for aflatoxin and fumonisin B1, respectively.

3. Segregation by size features

Aflatoxin contamination has been related to smaller grains and nuts in commodities lots (Dorner et al. 1989; Whitaker et al., 2005; Schatzki & Pan, 1996). Besides the high correlation between aflatoxin and size, infected grains and nuts can be more friable than not infected ones (Shotwell et al., 1974), therefore the handling of the product can generate fragments, as shown in figure 3, of infected material, and it can contribute to the total aflatoxin level of the lot (Meinders & Hurburg, 1993; Piedade et al., 2002). Therefore, segregation by size has been studied as a way to remove aflatoxin contamination in commodities lots. Generally, size sorting is carried out by using sieves with holes that allow small grains or nuts to pass through, while retaining larger ones. The size sorting process can involve different sieves with decreasing hole sizes. The process is primarily used to categorize commodities by grains and nuts in size, where the largest categories are more valued in the market, and to clean the product to improve the overall quality of the lot. Thus, aflatoxin reduction by size sorting is normally a secondary result.

In spite of this, some data have been reported about the correlation between size and aflatoxin levels, and the effect of processes based on size segregation in the aflatoxin levels of processed lots of commodities. Brekke et al. (1975) evaluated cleaning procedures which remove broken kernels and foreign materials in white and yellow maize lots and they could not observe satisfactory aflatoxin reduction. Cole et al. (1995) reported that using a farmer stock peanut the sizing and electronic color sorting process were responsible by 29 and 70% of the aflatoxin reduction. Piedade et al. (2002) investigated the aflatoxin segregation when a sieve of 4.5 mm of round-holes was used to sieve maize samples and they observed that the largest grain fraction had lower average levels (84.8 µg/Kg) than the smallest one (204.0 µg/Kg). However, due to weight participation of each fraction in the total sample weight, the contribution of the smallest fraction was lower than the largest, so the segregation by size would not be able to reduce the aflatoxin levels in the whole sample. Meinders & Hurburg (1993) also detected a concentration in the aflatoxin levels as decreasing maize fractions from 6.3 to 1.8 mm were analyzed. Schatzki & Pan (1996) showed a positive relation between small pistachio nuts and aflatoxin levels. Whitaker et al. (2005) evaluated the aflatoxin distribution among peanut size categories using 46 peanut mini-lots. A negative correlation between size and aflatoxin content was observed. The shelled peanuts showed an average contamination of 75.3 µg/Kg, before the sorting, and after the sorting

the six categories showed average contaminations of 42.5, 66.2, 93.6, 116.7, 105.1 and 133.6 $\mu\text{g}/\text{Kg}$. Only the two largest categories showed aflatoxin levels lower than the initial level.

Dowell et al. (1990) reported data about aflatoxin reduction when belt screen was used to screen unshelled peanuts to separate loose kernels and small pods. An average of 35% of reduction in the aflatoxin levels was observed when 17 lots were processed with belt screen. According to Dorner (2008), that type of device has been widely used by the USA peanut industry. Whitaker reported that the initial mean aflatoxin concentration of 73.7 $\mu\text{g}/\text{Kg}$ was reduced to means of 42.5 and 66.2 $\mu\text{g}/\text{Kg}$ in the large (named jumbo) and medium size categories of peanut, respectively, but was increased to 93.6, 105.1, and 133.6 $\mu\text{g}/\text{Kg}$ in the smaller categories number one, sound split, and oil stock categories, respectively.



Fig. 4. Broken maize grains that can be remove by size sorting

4. Segregation by density

Grains and nuts, in which fungal growth and insect attack occurred, can present lower density than sound ones (Kabak et al., 2006). This characteristic has been used to separate poor quality material in commodities. In addition, the possibility to remove poor quality material brings the possibility to reduce aflatoxin contamination in food lots, because normally, the aflatoxin contamination is concentrated in poor quality material. Research on aflatoxin segregation by differences in density has been carried out, e.g., in maize, peanuts, and Brazil nuts.

Huff (1980) obtained 60% of aflatoxin levels when buoyant maize in water was removed. Sucrose solutions could improve the aflatoxin reduction to 90%, as the concentration of

sucrose was increased up to 40%, but in this case 53% of maize was removed. Huff et al. (1982) also observed that flotation of maize in water and in 30% sucrose solution were efficient to segregate the aflatoxin contamination. Kirksey et al. (1989) studied the aflatoxin distribution in relation to peanut kernel density. They put 500 g of peanuts in 2000 mL of tapwater, and 15-30% of the kernel rose to the surface as buoyant kernels and they contained an average of 95% of total aflatoxin present in the samples. It was observed that kernels floated due to a hollow space inside them between cotyledons, which consisted in a reservoir of air to flotation, fungal growth, and aflatoxin production. Henderson et al. (1989) patented a procedure based on flotation of contaminated peanuts, but this procedure has not been widely used due to an additional drying step necessary after the flotation process. Gnanasekharan et al. (1992) found a negative correlation between aflatoxin content and density of peanut kernels, showing that kernels of low density have high probability to be contaminated. Steiner et al. (1992) reported that the weight of kernels in Brazil nuts evaluated was not a good indicator of aflatoxin contamination. Clavero et al. (1993) evaluated a method of flotation based on maize grain immersion in hydrogen peroxide. They observed a segregation of 90% in the initial aflatoxin contamination. The method was based on the catalase reaction with hydrogen peroxide. Clavero et al. (1993) demonstrated that *A. parasiticus* can produce catalase in peanut milk. Then, it was hypothesized that catalase produced by *A. parasiticus* would react with hydrogen peroxide and promote the formation of oxygen bubbles on the surface of the mold-infected kernels, causing their flotation.

5. Sampling procedures based on grain and nut types with high contamination probability

Grains and nuts, in which fungal growth and insect attack occurred, can present lower density than sound ones (Kabak et al., 2006). This characteristic has been used to separate poor quality material in commodities. In addition, the possibility to remove poor quality material brings the possibility to reduce aflatoxin contamination in food lots, because normally, the aflatoxin contamination is concentrated in poor quality material. Research on aflatoxin segregation by differences in density has been carried out, e.g., in maize, peanuts, and Brazil nuts.

Huff (1980) obtained 60% of aflatoxin levels when buoyant maize in water was removed. Sucrose solutions could improve the aflatoxin reduction to 90%, as the concentration of sucrose was increased up to 40%, but in this case 53% of maize was removed. Huff et al. (1982) also observed that flotation of maize in water and in 30% sucrose solution were efficient to segregate the aflatoxin contamination. Kirksey et al. (1989) studied the aflatoxin distribution in relation to peanut kernel density. They put 500 g of peanuts in 2000 mL of tapwater, and 15-30% of the kernel rose to the surface as buoyant kernels and they contained an average of 95% of total aflatoxin present in the samples. It was observed that kernels floated due to a hollow space inside them between cotyledons, which consisted in a reservoir of air to flotation, fungal growth, and aflatoxin production. Henderson et al. (1989) patented a procedure based on flotation of contaminated peanuts, but this procedure has not been widely used due to an additional drying step to be necessary after the flotation process. Gnanasekharan et al. (1992) found a negative correlation between aflatoxin content and density of peanut kernels, showing that kernels of low density have high probability to be contaminated. Steiner et al. (1992) reported that the weight of kernels in Brazil nuts evaluated was not a good indicator of aflatoxin contamination. Clavero et al. (1993) evaluated a method of flotation based on maize

grain immersion in hydrogen peroxide. They observed a segregation of 90% in the initial aflatoxin contamination. The method was based on the catalase reaction with hydrogen peroxide. Clavero et al. (1993) demonstrated that *A. parasiticus* can produce catalase in peanut milk. Then, it was hypothesized that catalase produced by *A. parasiticus* would react with hydrogen peroxide and promote the formation of oxygen bubbles on the surface of the mold-infected kernels, causing their flotation.

In Brazil, the animal production industry, mainly poultry sector, has used gravimetric tables, a machine that segregate maize grains in high and low density fractions, to obtain mycotoxin segregation in maize. The high density fractions, which contain grains with low probability of mycotoxin contamination, is intended to make feeds for younger poultry, which are more susceptible to mycotoxin effects. The low density fraction, which has high probability of mycotoxin contamination, is intended to make feed for other poultry.

6. Sampling procedures based on grain and nut types with high contamination probability

The uneven distribution of aflatoxin contaminated grains and nut inside a lot normally represents a problem for measuring the true average level of aflatoxin contamination. However, some researchers have tried to take advantage of the distribution concentrated in few grains and nuts which can present different visual, optical, or physical characteristics in relation to sound ones that are not contaminated. From the analysis of samples containing only poor quality material, they have tried to improve the sampling plans efficiency to indicate lots which are above or under an established limit of acceptance for aflatoxin contamination.

Whitaker et al. (1998) studied the possibility to measure the aflatoxin contamination of farmers' stock peanuts by measuring the contamination in various peanut-grade categories. It was observed that best indicator for the aflatoxin concentration in the lot was the aflatoxin mass combined in the Loose Shelled Kernels (LSK), Damaged Kernels (DAM), and Other Kernels (OK). Whitaker et al. (1999) evaluated the performance of sampling plans based on the measurement of aflatoxin contamination in peanut-grade categories collected from a 2 Kg sample of the farmers' stock peanut lots, and establishing an acceptance limit of 50 µg/Kg. They observed that sampling plans based on combined mass of aflatoxin in LSK, DAM, and OK gave the best operating curve. Johansson et al. (2006) studied the possibility to predict aflatoxin in maize lots using poor-quality grade components. The aflatoxin mass combined in Damaged Kernels (DAM), and in Broken Kernel and Foreign Material was highly correlated with aflatoxin contamination in the lot, so they suggested that the measured aflatoxin mass combined with grade components could be used as a screening method to predict aflatoxin in maize lots.

Otherwise, Gloria et al. (2010) compare the performance of a sampling plan based on measuring the aflatoxin contamination in combined types of damaged grain maize, which was withdrawn from an 1 Kg sample of maize, with a sampling plan based on measuring aflatoxin in all types of grain (sound and damaged) in a sample test of ca. 5 Kg. The best operating curve was obtained by the sampling plan based on a 5 Kg test sample.

7. Conclusions

Several technologies for aflatoxin contamination segregation have been proposed in the scientific literature, but just some are currently used by the industry. Some processes have

been used to improve the overall quality of commodities, and the reduction of aflatoxin is just a consequence and not the objective. The electronic color, in the visible or near infra-red wavelengths, alone or combined with other technology of sorting, is the technology most widely used by the industry and which has shown a great improvement of modern optical possibilities and consequently improve aflatoxin remotion.

8. References

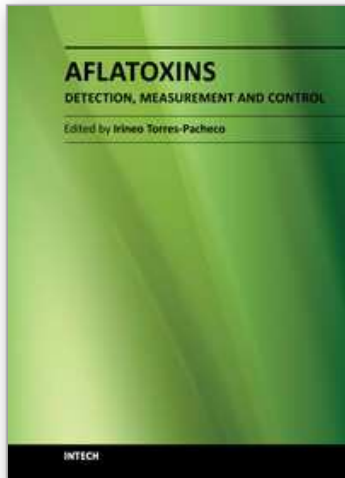
- Ayres, J.L. (1977). Aflatoxins in Pecans: Problems and Solutions. *Journal of the American Oil Chemists' Society*, Vol. 54, No 3, pp A229-A230
- Benford, D.; Leblanc, J.C. & Setzer, R.W. (2010). Application of the margin of exposure (MoE) approach to substances in food that are genotoxic and carcinogenic. Example: Aflatoxin B1 (AFB1). *Food Chemical Toxicology*, Vol. 48, pp 534-541
- Brown, G.H. (1984). The distribution of total aflatoxin levels in composited samples of peanuts. *Food Technology in Australia*, Vol. 36, pp 128-130
- Campbell, B.C.; Molyneux, R.J. & Shatzki, T. (2003). Current Research on Reducing Pre-and Post-harvested Aflatoxin Contamination of U.S. Almond, Pistachio, and Walnut. *Journal of Toxicology TOXIN REVIEWS*, Vol. 22, No.2 & 3, pp 225-266
- Campbell, B.C.; Molyneux, R.J. & Shatzki, T. (2006). Advances in Reducing aflatoxin Contamination of U.S. Tree Nuts, In: *Aflatoxin and Food Safety*, Abbas, H.K. (Ed.), 483-516, CRC Press, Boca Raton, FL, USA
- Clavero, M.R.S.; Hung, Y.; Beuchat, L.R & Nakayama, T. (1992). Catalase content in peanut milk as affected by growth by *Aspergillus parasiticus*. *Journal of Food Protection*, Vol 56, pp 55-57
- Clavero, M.R.S; Hung, Y.C.; Beuchat, L.R.; Nakayama, T. (1993). Separation of aflatoxin-contaminated kernels from sound kernels by hydrogen peroxide treatment. *Journal of Food Protection*, Vol. 56, No.2, pp 130-136, 156
- Cole, R.J.; Dorner, J.W. & Holbrook, C.C. (1995). Advances in mycotoxin elimination and resistance. In: *Advances in Peanut Science*, Pattee, H.E. & Stalker, H.T. (Eds.), 456-474, American Peanut Research Educational Society, Stillwater, OK, USA
- Cucullu, A.F.; Lee, L.S.; Mayne, R.Y. & Goldblatt, L.A. (1966). Determination of aflatoxin in individual peanuts and peanut sections. *Journal of the American Oil Chemists' Society*, Vol. 43, No. 2, pp 89-92
- Cucullu, A.F.; Lee, L.S. & Pons, W.A. Relationship of physical appearance of individual damaged cottonseed to aflatoxin content. *Journal of the American Oil Chemists' Society*, Vol. 54, No.3, pp A235-A237
- De Mello, F.R. and Scussel, V. Development of Physical and Optical Methods for in-shell Brazil nuts Sorting and Aflatoxin. *Journal of Agricultural Science*, Vol. 1, No. 2, pp 3-14
- Dickens, J. & Welty, R.J. (1975). Fluorescence in pistachio nuts contaminated with aflatoxin. *Journal of American Oil Chemists' Society*, Vol.52, pp 448-450
- Dickens, J. & Whitaker, T.B. (1975). Efficacy of Electronic Color Sorter and Hand Picking to Remove Aflatoxin Contaminated Kernels from Commercial Lots of Shelled Peanuts. *Peanut Science*, Vol. 2, No.2, pp 45-50
- Dickens, J.W. & Whitaker, T.B. (1986). Sampling and Sample Preparation Methods for Mycotoxin Analysis, In: *Modern Methods in the Analysis and Structural Elucidation of Mycotoxins*, Cole, J. (Ed.), 29-49, Academic Press, Inc., Oakland, FL, USA

- Diener, U.L.; Cole, R.J.; Sanders, T.H.; Payne, G.; Lee, L.S. & Klich, M.A. (1987). Epidemiology of Aflatoxin formation by *Aspergillus flavus*. *Annual Review of Phytopathology*, Vol.25, No. , pp 249-270
- Dorner, J.W. (2008). Management and prevention of mycotoxins in peanuts. *Food Additives and Contaminants*, Vol. 25, No. 2, pp 203-208.
- Dowell, F.E.; Dorner, J.W.; Cole, R.J. & Davidson, J.I. (1990). Aflatoxin reduction by screening farmers stock peanuts. *Peanut Science*, Vol. 17, pp 6-8
- Escher, F. (1974). Mycotoxin problems in the production and processing of peanuts and pecans in the USA. *Lebensmitt Wiss Technol*, Vol. 7, pp 255
- Farsaie, A.; McClure, W.F. & Monroe, R.J. (1981). Design and development of an automatic electro-optical sorter for removing BGY fluorescent pistachio nuts. *Transactions of the ASAE*, Vol. 24, No.5, pp 1372-1375
- Frisvald, J.C.; Skoube, P. & Samson, R.A. (2005). Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B1, sterigmatocystin and 3-O-methylsterigmatocystin, *Aspergillus rambellii* sp. nov. *Systematic and Applied Microbiology*, Vol. 28, pp 442-453
- Fuller, G.; Spooncer, W.W.; King, A.D.; Shade, J. and Mackey, B. (1977). Survey of aflatoxins in California tree nuts. *Journal of the American Oil Chemists' Society*, Vol. 54, No.3, pp A231-A234
- Galvez, F.C.; Francisco, M.L.D.L.; Lustre, A.O. & Resurrecion, A.V.A. (2002). *Controlo f Aflatoxin in raw peanuts through proper manual sorting*. United States Agency for International Development, Peanut Collaborative Research Support Program, Project 04 (USA and Philippines), Monograph Series No. 3, Retrieved from <http://168.29.148.65/images/pdfs/reports/monograph3.PDF>
- Gloria, E.M.; Ciacco, C.F.; Lopes-Filho, J.F.; Ericsson, C. & Zocchi, S.S. (2006). Distribution of aflatoxin contamination in maize samples. *Ciência e Tecnologia de Alimentos*, Vol. 24, No. 1, PP 71-75
- Gloria, E.M.; Janeiro, V.; Abdallah, M.F.I.; Borges, F.C.; Bertelli, M.; Gasparotto, B.; Tomazella, T.; Calori-Domingues, M.A. & Zocchi, S.S. (2010). Aflatoxin contamination estimate of corn truck loads based on different quality kernels. *Proceedings of 124th AOAC Annual Meeting & Exposition*, Orlando, FL, USA, September, 2010
- Giesbrecht, F.G. & Whitaker, T.B. (1998). Investigations of the Problems of Assessing Aflatoxin Levels in Peanuts. *Biometrics*, Vol. 54, June, pp 739-753
- Gnanasekharan, M.S.; Chinnan, M.S. & Dorner, J.W. (1992). Methods for Characterization of Kernel Density and Aflatoxin Levels of Individual Peanuts. *Peanut Science*, Vol.19, pp 24-28
- Gürses, M. Mycoflora and Aflatoxin Content of Hazelnuts, Walnuts, Peanuts, Almonds and Roasted Chickpeas(LEBLEBI) Sold in Turkey. (2006). *International Journal of Food Properties*, Vol. 9, No. 3, pp 395-399
- Hadavi, E. (2005). Several physical properties of aflatoxin contaminated pistachio nuts: Application of BGY fluorescence for separation of aflatoxin contaminated nuts. *Food Additives and Contaminants*, Vol. 22, No. 1, pp 1144-1153
- Hirano, S.; Okawara, N. & Narazaki, S. (1998). Near Infra red Detection of Internally Mold Nuts. *Bioscience, Biochemistry, and Biotechnology*, Vol. 62, No.1, pp 102-107
- Henderson, J.C.; Kreutcher, S.H.; Schmidt, A.A. & Hagen, W.R. (1989). Flotation separation of aflatoxin contaminated grain or nuts. U.S. Patent No. 4.795.651.
- Huff., W.E. (1980). A physical method for the segregation of aflatoxin-contaminated corn. *Cereal Chemistry*, Vol. 57, No. 4, pp 236-238

- Johansson, A.S.; Whitaker, T.B.; Hagler, W.M.; Jr, Bowman, D.T.; Slate, A.B. & Payne, G. (2006). Predictin Aflatoxin and Fumonisin in Shelled Corn Lots Using Poor-Quality Grade Components. *Journal of AOAC International*, Vol. 89, No.2, pp 433-440
- Kabak, B.; Dobson, A.D.W. & Var, I. (2006) Strategies to Prevent Mycotoxin Contamination of Food and Animal Feed: A Review. *Critical Reviews in Food Science and Nutrition*, Vol. 46, pp 593-619
- Knutti, R. & Schlatter, C. (1978). Problems of assessing aflatoxin in peanut-Proposal for a sampling and analysis plan for control of imports. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*
- Knutti, R. & Schlatter, C. (1982). Distribution of aflatoxin in whole peanut kernels, sampling plans for small sample. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A*, Vol. 174, No. 2, pp 122-128
- Kumar, M. & Agarwal, V.K. (1997). Fungi detected from different types of seeds discoloration in maize. *Seed Research*, Vol. 25, pp 88-91
- Meinders, B.L. & Hurburg Jr., C.R. (1993). Properties of corn screenings. *Transaction of the ASAE*, Vol. 36, pp 811-819
- Molyneux, R.J.; Mahoney, N. & Campbell, B.C. (2007). Mycotoxins in edible tree nuts. *International Journal of Food Microbiology*, Vol. 119, No. , pp 72-78
- Moss, M. Risk assessment for aflatoxins in foodstuffs. (2002). *International Biodeterioration & Biodegradation*, Vol. 50, No.3-4, pp 137-142
- Van Egmond, H.P.; Schothorst, R.C. & Jonker, M.A. (2007). Regulations relating to mycotoxins in food. *Analytical and Bioanalytical Chemistry*, Vol. 389, pp 147-157
- Ozay, G.; Seyhan, F.; Yilmaz, A. Whitaker, T.B.; Slate, A.B. & Giesbrecht, F. (2007). Sampling Hazelnuts for Aflatoxin: Effect of Sample Size and Accept/Reject Limit on Reducing the Risk of Misclassifying Lots. *Journal of AOAC International*, Vol. 90, No. 4, pp 1028-1035
- Pearson, T. (1996). Machine vision system for automated detection of stained pistachio nuts. *Lebensmittel-Wissenschaft und-Technologie*, Vol. 28, No.6, pp 203-209
- Pearson, T.C. & Schatzki, T.F. (1998). Machine Vision System for Automated Detection of Aflatoxin-Contaminated Pistachios. *Journal of Agricultural Food Chemistry*, Vol. 46, No. 6, pp 2248-2252
- Pearson, T.C.; Wicklow, D.T.; Maghirang, E.B.; Xie, F. & Dowell, F.E. (2001). Detecting Aflatoxin in Single Corn Kernels by Transmittance and Reflectance Spectroscopy. *Transaction of the ASAE*, Vol. 44, No. 5, pp 1247-1254
- Pearson, T.C.; Wicklow, D.T. and Pasikatan, M.C.(2004). Reduction of aflatoxin and fumonisin contamination in yellow corn by high-speed dual-wavelength sorting. *Cereal Chemistry*, Vol. 81, pp 490-498
- Pelletier, M. J.; Spetz, W. L. and Aultz, T. R. (1991). Fluorescence sorting instrument for the removal of aflatoxin from large numbers of peanuts. *Review of Scientific Instruments*, Vol.62, No.8, pp1926-1931
- Piedade, F.; Fonseca, H.; Gloria, E.M.; Calori-Domingues, M.A.; Piedade, S.M.S. & Barbin, D. (2002). Distribution of Aflatoxins in contaminated corn fractions segregated by size. *Brazilian Journal of Microbiology*, Vol. 33, pp 12-16
- Pomenranz, Y. (1992). Biochemical, functional, and nutritive changes during storage. In: *Storage of Cereal Grains and Their Products*, Sauer, D.B. (Ed.), 55-141, 4th ed., American Association of Cereal Chemists, St. Paul, Minnesota, USA
- Robin, Y; Chiou, Y; Wu, P.Y. & Yen, Y.H. (1995). Color sorting of lightly roasted and deskinne peanut kernels to diminish aflatoxin and retain the processing potency. *Developments in Food Science*, Vol. 37, pp 1533-1546

- Schade, J. & King, Jr. A.D. (1984). Fluorescence and Aflatoxin Content of Individual Almond Kernels Naturally Contaminated with Aflatoxin. *Journal of Food Science*, Vol. 49, No. ,pp 493-497
- Schade, J.E.; McGreevy, K.; King, Jr. A.D.; Mackey, B. & Fuller, G. (1975). Incidence of Aflatoxin in California Almonds. *Applied Microbiology*, Vol. 29, No. 1, pp 48-53.
- Sharkey, A.J.; Roch, O.G. & Coker, R.D. (1994). A case-study on the development of a sampling and testing protocol for aflatoxin levels in edible nuts and oil-seeds. *The Statistician*, Vol. 43, No. 2, pp 267-275
- Shatzki, T.F. (1995). Distribution of Aflatoxin in Pistachios. 2. Distribution in Freshly Harvested Pistachios. *Journal of Agricultural and Food Chemistry*, Vol.43, No.6, pp 1566-1569
- Shatzki, T. & Pan, J. (1996). Distribution of Aflatoxin in Pistachios. 3. Distribution in Pistachio Process Streams. *Journal of Agricultural and Food Chemistry*, Vol. 44, No. 4, pp1076-1084
- Shotwell, O.L.; Goulden, M.L. and Hesseltine, C.W. (1974). Aflatoxin: Distribution in contaminated corn. *Cereal Chemistry*, Vol. 51, pp 492-499
- Shotwell, O.L. & Hesseltine, C.W. (1981). Use of bright greenish yellow fluorescence as a presumptive test for aflatoxin in corn. *Cereal Chemistry*, Vol.58, No.2, pp124-127
- Sommer, N.F.; Buchanan, J.R. & Fortlage, R.J. (1986). Relation of early splitting and tattering of pistachio nuts to aflatoxin in the orchard. *Phytopathology*, Vol. 76, pp 692-694
- Steiner, W.; Rieker, H. & Battaglia, R. (1988). Aflatoxin contamination in dried figs: distribution and association with fluorescence. *Journal of Agricultural and Food Chemistry*, Vol.36, No., pp 88-91
- Steiner, W.E.; Brunschweiler, K.; Leimbacher, E. and Schneider, R. (1992). Aflatoxins and Fluorescence in Brazil Nuts and Pistachio Nut. *Journal of Agricultural and Food Chemistry*, Vol. 40, No.12, pp 2453-2457
- Stoloff, L.; Campbell, A.D.; Becwith, A.C.; Neshiem, S.; Winbrush, J.S.Jr.; & Fordham, A.M. (1969). Sample preparation for aflatoxin assay. The nature of the problem and approaches to a solution. *Journal of the American Oil Chemists' Society*, Vol. 46, pp 678-684
- Tiemstra, P.J. (1969). A study of the variability associated with sampling peanuts for aflatoxin. *Journal of the American Oil Chemists' Society*, Vol. 46, pp 667-672
- Tyson, T.W. & Clark, R.L. (1974). An investigation of the fluorescent properties of aflatoxin infected pecans. *Transaction of the ASAE*, Vol. 17, No. 5, pp 942-945
- Wacowicz, E. (1991). Changes of chemical grain components, especially lipids, during their deterioration by fungi. In: *Cereal Grain: Mycotoxins, Fungi and Quality in Drying and Storage*, Chelkowski, J. (Ed.), 259-280, Elsevier, Amsterdam, Netherlands
- Waibel, Von J. (1977). Stichprobengröße für die Bestimmung von Aflatoxin in Erdnüssen. *Deutsche Lebensmittel-Rundschau*, Vol 73, No. 11, pp 353-357
- Whitaker, T.B. & Wiser, E.H. (1969). Theoretical Investigations Into the Accuracy of Sampling Shelled Peanuts for Aflatoxin. *Journal of the American Oil Chemists' Society*, Vol. 46, July , pp 377-379
- Whitaker, T.B.; Dickens, J.W.; Monroe, R.J. & Wiser, E.H. (1972). Comparison of the Observed Distribution of Aflatoxin in Shelled Peanuts to the Negative Binomial Distribution. *Journal of the American Oil Chemists' Society*, Vol. 9, No. 10, pp 590-593
- Whitaker, T.B.; Giesbrecht, F.G.; Hagler, Wu,J.; Hagler, W.M.& Dowell,F.E. (1994). Predicting the Distribution of Aflatoxin Test Results from Farmers' Stock Peanuts. *Journal of Association of Official Analytical Chemists*, Vol.77, pp 659-666

- Whitaker, T.B.; Giesbrecht, F.G. & Wu, J. (1996). Suitability of Several Statistical Models to Simulate Observed Distribution of Sample Test Results in Inspection of Aflatoxin-Contaminated Peanut Lots. *Journal of AOAC International*, Vol. 79, No. 4, pp 981-988
- Whitaker, T.B. (1997). Efficiency of the Blanching and Electronic Color Sorting Process for Reducing Aflatoxin in Raw Shelled Peanuts. *Peanut Science*, Vol. 24, pp 62-66
- Whitaker, T.B.; Hagler, W.M.Jr.; Giesbrecht, F.G.; Domer, J.W.; Dowell, F.E. & Cole, R.F. (1998). Estimating Aflatoxin in Farmers' Stock Peanut Lots by Measuring Aflatoxin in Various Peanut-Grade Components. *Journal of AOAC International*, Vol 81, No. 1, pp 61-67
- Whitaker, T.B.; Hagler, Jr. W.M. & Giesbrecht, F. (1999). Performance of Sampling Plans To Determine Aflatoxin in Farmers' Stock Peanut Lots by Measuring Aflatoxin in High-Risk-Grade Components. *Journal of AOAC International*, Vol 82, No.2, pp 264-270
- Whitaker, T.B.; Dorner, J.W.; Lamb, M. & Slate, A.B. (2005). The effect of sorting farmers' Stock Peanut by Size and Color on Partitioning Aflatoxin into Various Shelled Peanut Grade Sizes. *Peanut Science*, Vol. 32, pp 103-118
- Whitaker, T.B.; Slate, A.B. & Johansson, A.S. (2005b). Sampling feeds for mycotoxin analysis. In: *The Mycotoxin Blue Book*, Diaz, D.E. (Ed.), 1-23, Nottingham University Press, Thrumpton, Nottingham, United Kingdom
- Whitaker, T.B.; Slate, A.; Adams, T.B.J.; Jacobs, M. & Gray, G. (2010). Correlation Between Aflatoxin Contamination and Various USDA Grade Categories of Shelled Almonds. *Journal of AOAC International*, Vol 93, No. 3, pp 943
- Wicklow, D.T. & Pearson, T.C. (2006). Detection and removal of single mycotoxin contaminated maize grains following harvest. *Proceedings of 9th International Working Conference on Stored Product Protection*, Campinas-SP, Brasil, October, 2006
- Wicklow, D.T. (1999). Influence of *Aspergillus flavus* Strains on Aflatoxin and Bright Greenish Yellow Fluorescence of Corn Kernels. Vol. 83, No. 12, pp 1146-1148
- Wild, C.P. & Gong, Y.Y. (2010). Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis*, Vol. 31, No.1, pp 71-82
- Wilson, D. (1989). Analytical method for aflatoxin in corn and peanuts. *Archives of Environmental Contamination and Toxicology*, Vol. 18, No. 3, 308-314
- Yao, H.; Hruska, Z.; Kincaid, R.; Brown, R.; Cleveland, T. & Bhatnagar, D. (2010). Correlation and classification of single kernel fluorescence hyperspectral data with aflatoxin concentration in corn kernels inoculated with *Aspergillus flavus* spores. *Food Additives and Contaminants*, Vol. 27, No. 5, pp 701-709



Aflatoxins - Detection, Measurement and Control

Edited by Dr Irineo Torres-Pacheco

ISBN 978-953-307-711-6

Hard cover, 364 pages

Publisher InTech

Published online 21, October, 2011

Published in print edition October, 2011

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.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Eduardo Micotti da Gloria (2011). Aflatoxin Contamination Distribution Among Grains and Nuts, Aflatoxins - Detection, Measurement and Control, Dr Irineo Torres-Pacheco (Ed.), ISBN: 978-953-307-711-6, InTech, Available from: <http://www.intechopen.com/books/aflatoxins-detection-measurement-and-control/aflatoxin-contamination-distribution-among-grains-and-nuts>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
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

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen