METHODS OF CONTROLLING AFLATOXINS IN FOODS AND FEEDSTUFFS: AN UPDATE

W.O. Ellis, PhD
J.H. Oldham, MSc.
Dept. Of Biochemistry, U.S.T., Kumasi. Ghana.

J.P. Smith, PhD,
B.K. Simpson, PhD.
Dept. Of Food Science and Agricultural Chemistry, McGill University, Montreal. Canada.

ABSTRACT

Aflatoxins are mycotoxins produced by species of Aspergilli, specifically Aspergillus flavus and Aspergillus parasiticus. These molds are ubiquitous in nature due to the susceptibility of most organic matter to support their growth and aflatoxin production. Aflatoxins are of great concern worldwide due to their biochemical and biological effects on living organisms. In this article, the various methods of controlling aflatoxins and their limitations, including the novel techniques of active atmosphere modification are reviewed. Future areas of potential research involving the use of natural antimicrobial agents which would be of immense advantage to developing countries such as Ghana are also discussed.

***KEYWORDS**: Aflatoxins, mycotoxins, A. flavus, A. parasiticus, Chitosan.*

INTRODUCTION

Aflatoxins are mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. These molds are ubiquitous in nature and therefore are capable of growing on a wide variety of substrates and under a variety of environmental conditions [1]. Molds are beneficial to mankind in several ways, such as the production of cheese, bread, antibiotics, vitamins, enzymes, glycerol, alcohol and organic acid fermentations [2]. However, many mold species such as *Aspergillus flavus* and *Aspergillus parasiticus* are harmful due to the production of harmful substances such as sterigmatocystin [3], aspergillic acid [4], aspertoxin [5] and aflatoxins [6]. In general, there are 18 different types of aflatoxins of which aflatoxins B_1, B_2, G_1, G_2, M_1 and M_2 are the most common [7]. Aflatoxins like other known mycotoxins are relatively small organic compounds. They are not infectious, since they are not living organisms and are also not antigenic. Aflatoxins do not accumulate in fat tissues of the body as do many pesticides [8], however they have been found to be carcinogenic, mutagenic, teratogenic, hepatotoxic and show other symptoms which have all been classified as aflatoxicosis. Aflatoxins also affect the biochemical systems in an organism such as carbohydrate, lipid, protein and nucleic acid metabolisms. These effects arise from the ingestion of food and feedstuff contaminated with aflatoxins [9].

The occurrence of aflatoxins is worldwide due to the ubiquitous nature of the molds and the susceptibility of most organic matter to support their growth and toxin production. An international survey carried out to assess the extent of aflatoxin contamination during the period 1976 to 1983 by 16 countries involving grains and nuts of major importance, showed that most of the grains monitored had aflatoxin levels above the regulatory level of 5-20 ug/kg for foods. However, the level of aflatoxin in commodities varied from year to year and from region to region even in the same country [10]. The high level of aflatoxin and in general mycotoxin contamination worldwide can be viewed in two ways, either through direct contamination or indirect contamination. Direct high level contamination results from the inability of the producer nation to effectively monitor and control aflatoxin levels and indirect high level contamination arise from the importation of contaminated products by consumer nations from producer nations without effective monitoring and control systems or programmes. Studies indicate that intensified efforts by both producer and consumer nations are needed to develop and maintain control in the worldwide supply of commodities [9]. Several methods of control have been used both at the laboratory and industrial scale levels with the aim of preventing product contamination by aflatoxigenic molds or making products aflatoxin-free for consumption. These control methods can be grouped into two;

(a) Prevention of mold growth and contamination and
(b) detoxification of contaminated products.

Of these two, unquestionably prevention is the best method of control especially for developing countries such as Ghana. However, in the presence of contamination there is no other option but to decontaminate bearing in mind the hazard associated with the toxin

W.O. Ellis

I H. Oldham

J.P. Smith

B.K. Simpson

on ingestion.

Detoxification processes involve degrading, destroying, or inactivating aflatoxins in commodities by physical, chemical or biological methods. Physical detoxification involves extraction (solvent), heat inactivation, irradiation and adsorption.

Extraction

Solvent extraction is one of the most effective means of removing aflatoxins from a contaminated product. Solvents used for extraction include 95% ethanol, 90% aqueous acetone, 80% isopropyl alcohol, 90% aqueous acetonitrile, hexane-ethanol and hexane-methanol. This method is not applied on a commercial scale but mainly used at the laboratory scale level. Studies show that with solvent extraction essentially all aflatoxins in most products can be removed with little likelihood of forming toxic by-products or reduction in protein content or nutritional quality of the meal [11]. The major disadvantages associated with the extraction methods are the cost of additional processing, the need for special extraction and solvent removal equipments, loss of some nutrients from residual meals, mainly carbohydrates, disposal of extracts are economic considerations, and it is also time consuming and the final products formed are in many ways not suitable for human consumption [9].

Heat (inactivation temperatures)

Aflatoxins are relatively heat stable and are only inactivated at temperatures as high as 250°C. Therefore, thermal inactivation procedures result in modest reduction in aflatoxin levels [12, 13]. Dry heat such as roasting temperatures approaching 250°C are very effective in the degradation of the aflatoxins [14]. Roasting techniques have been found to result in a 45-83% reduction in aflatoxin content in peanuts, depending on the conditions of roasting and the initial level of aflatoxin [15]. The moisture content of the heated product and the increase in pressure resulting from heating increases the amount of toxin destroyed [2]. This technique has also been applied to reduce aflatoxin levels in other commodities such as pecans and corn [11]. Pluyer et al. (1987) [16] in their studies on peanuts using both oven and microwave roasting also concluded that the above procedures were effective in destroying about 40 to 61% of aflatoxin in naturally contaminated peanuts. Although heat treatments lower aflatoxin levels, it is not an economically viable process since it affects the nutritive value of the product.

Use of radiation

Aflatoxins are sensitive to ultra-violet (UV) radiation. However, the use of UV irradiation as an effective means of detoxification is still questionable. Irradiation does not destroy aflatoxins easily partly due to (i) the small size of the toxin molecules and, (ii) partly due to the protective effect of the complex food media [17]. Therefore, aflatoxins are not generally affected by irradiation directly but in the presence of water, radiolysis occurs resulting in free radical formation which then reacts with the aflatoxins [18]. Gamma radiations of dose 1 Mrad has been used with some success in destroying molds at a substrate water content of 22%. Sometimes, the dose level required to effectively destroy aflatoxins would also destroy the nutritive value of the product [19]. The disadvantages of the irradiation technique are its inability to destroy aflatoxins at low or intermediate doses, its destruction of the nutritive value of the food product at high doses and the probability of high doses resulting in radioactivity.

Adsorption

The use of chemisorbent substances have been evaluated as potential methods of decontamination using the principle of adsorption. Studies have shown that bentonite, a clay with adsorptive properties has the full potential of removing almost all the aflatoxins present in a solution. Masimango et al. (1978) [20] observed that aflatoxin B_1 in solution was adsorbed by bentonite when it was added to the solution. Removal of the bentonite resulted in the removal of almost all of the aflatoxins present. The ability of bentonite to adsorb and retain aflatoxin was dependent on the particle size and the degree of heat treatment. Similar studies by Marth and Applebaum [18] resulted in the adsorption of aflatoxin M_1 from milk. However, much more research is required to determine the practical application of using bentonite for aflatoxin in milk and other fluid products. Other studies have been done on other chemical substances such as alumina, silica and aluminosilicate based on their adsorptive properties. From these evaluations, hydrated sodium calcium aluminosilicate (HSCAS) has been found to have a high affinity for aflatoxin B_1 through the formation of a stable association with aflatoxin B_1 [21, 22]. Phillips et al. (1988) [21] in their studies using HSCAS reported that, in vitro, HSCAS adsorbed more than 80% of the available aflatoxin B_1 from aqueous solutions with very little desorption (less than 10%) occuring, even in a series of eluotropic solvents. *In vivo* studies, using one day old male leghorn chicks also showed that HSCAS had protective effects on the gross hepatic changes arising from aflatoxin B_1. Therefore, it was suggested that HSCAS could modify the toxicity of aflatoxin B_1 in chickens through a sequestering effect resulting in reduced bioavailability of aflatoxin B_1. This technique seems very economical however, more research is required to determine the practical application of using the technique for other fluid products. The other disadvantage of this technique is that, it is only suitable for aqueous systems.

Chemical detoxification appears to be a promising method of removing aflatoxin from food commodities. This method involves acidification, alkalination and oxidation. A wide range of chemicals have been studied as degradative reagents for aflatoxins. Among these are acids, bases (alkali) and oxidizing agents. Although these chemicals show promise in the control of aflatoxins in both food and feedstuff, data supporting the

efficacy and safety of the use of most of these chemicals are lacking.

Acids destroy aflatoxins especially B_1 and G_1 by converting them to the corresponding hemiacetals, B_{2a} and G_{2a} through the incorporation of water [23]. Usually strong acids are very effective, however, notwithstanding their effectiveness, there are certain disadvantages to this technique; (i) the reaction with acids is very drastic and changes the properties of the final product, (ii) acids have little effect on aflatoxin B_2 and G_2, (iii) there is also the cost of additional processing to remove reagents and (iv) the final product formed is not suitable for human consumption.

Alkalination

Alkalination or the use of bases has been found to be very effective and relatively inexpensive for degrading aflatoxins. Alkaline reagents commonly used are sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), ammonia (NH_3), methylamine and ethylamine [24, 25]. Of all these, ammonia (ammoniation) is the most effective [26] and is being used on a large scale in the U. S. A., France, Brazil, Mexico, Sudan and Senegal [27]. The reaction occurs at high temperatures and pressures and reduction is in the range of 95% to 98% for aflatoxins in peanut meal [28]. In recent times, this technique is being practised at ambient temperature and atmospheric pressure to degrade aflatoxin [29]. The mechanism is based on the opening of the lactone ring of aflatoxin B_1 by ammonia, forming an ammonium salt with the hydroxyacid. The *B*-keto acid formed is decarboxylated due to the high temperatures and pressure to give aflatoxin D_1. This technique is mainly used for animal feeds [28]. The set-backs with this technique are (i) its production of off-flavours and off-odours, (ii) lowering of the protein efficiency ratio, (iii) volatile bases like the amines producing toxic end products, (iv) the residual product formed being only suitable for feedstuff whilst (v) expensive equipments are also needed for large scale treatments especially large scale set-ups using high temperatures and pressures.

Oxidizing agents

Several oxidizing agents attack the terminal double bonds of the dihydrofuran ring of aflatoxins B_1, G_1, and M_1. Aflatoxins B_2, G_2 and M_2 are more resistant due to lack of a terminal double bond [30]. Some of the oxidizing agents are oxygen, ozone, air and hydrogen peroxide (H_2O_2). H_2O_2 brings about 97% destruction of aflatoxin in defatted peanut meals [31]. The disadvantages of this technique are, (i) it occurs under high temperatures and is time consuming, (ii) it results in drastic lysine reduction, (iii) the use of ozone results in decrease in protein efficiency ratio and (iv) it is unable to destroy aflatoxin B_2.

Biological detoxification involves the use of microorganisms but this technique is still at the laboratory stage. Aflatoxin degradation involving microbes vary depending on the type of microorganism. Table 1 shows a list of microbes which can metabolize aflatoxins B_1, G_1, and M_1 to aflatoxicol and other compounds.

TABLE 1. : Microorganisms converting aflatoxin B_1 to aflatoxicol

Flavobacterium aurantiacum	Aspergillus niger
Corynobacterium rubrum	Mucor ambigus
Trichoderma viride	Dactylium denroides
Mucor alternans	Rhizopus stolonifer
Absidia repens	Mucor griseo-cyanus
Rhizopus oryzae	Rhizopus arrhizus [16]

Flavobacterium aurantiacum NRRL B-184 seems to be the most effective [32]. The degradation is thought to occur through enzymatic activity and that these enzymes produce end products or by-products which react with aflatoxin. Peroxidase was speculated to be one such enzyme, since it catalyses the decomposition of hydroperoxides to produce free radicals [33], which then react with aflatoxins. *Lactobacillus acidophilus*, *L. bulgaricus* and *L. plantarum* have also been shown to prevent mold growth as well as degrade aflatoxins. The prevention of mold growth was attributed to pH effect [34]. The set-backs with this process are (i) the aflatoxicol formed is a toxic product, (ii) process is time consuming and incomplete, (iii) and process is inhibited by microbial competition. The technique still requires further studies.

Generally, a successful detoxification process should satisfy the following criteria;

1. It must be economical,
2. It must be capable of removing all traces of aflatoxin leaving no deleterous residues,
3. It must not impair the nutritional quality of the product,
4. It must not alter significantly the technologi cal properties of the product and finally,
5. It must if possible, destroy fungal spores [27]. However, most of these detoxification techniques do not satisfy the above criteria.

B. Prevention of mold growth and contamination.

This approach seems to be one of the most feasible and practical for controlling aflatoxins, especially in developing countries such as Ghana. This approach can be achieved through one of the following methods;

a. Improved farm management

b. Antifungal agents

c. Rapid screening techniques

d. Environmental control.

Improved Farm Management

Studies have shown that fungal attack occurs in the field therefore, contamination of plants with aflatoxin occurs in growing plants in the field [35, 36, 37]. This has been observed in peanuts, corn and cottonseed during stress conditions [38, 39].

To prevent this, there is the need for good farm management practices such as,

a. the use of sound fungus-free seeds for planting,

b. controlling insects and plant diseases,

c. controlling irrigation practices,

d. harvesting plants at maturity,

e. good handling of harvesting equipments to prevent crop damage, and finally

f. good storage facilities with sound environmental conditions can all be used to prevent contamination of aflatoxin producing molds [11]. This preventive approach to aflatoxin control would be very suitable to Ghana and needs to be given a major attention. There are certain limitations to the effective implementation of these procedures. These are; (i) they are very expensive and therefore are most suitable for large scale farming and (ii) in most developing countries such as Ghana, illiteracy is a major set-back. However, with the government's effort to improve literacy through the Non-functional literacy programmes efforts should be made to make this approach a priority in the food production sector.

Antifungal agents

Certain chemical compounds and short chain fatty acids inhibit fungal growth. Propionic acid and salts of sorbic acid have been found to be very effective at acid pH against *A. flavus* and *A. parasiticus* [40, 41]. The compound dichlorvos has also been found to inhibit aflatoxin biosynthesis by *Aspergillus parasiticus* even though it has no effect on fungal growth [42]. The effect of antifungal substances depends on various factors such as; (i) the composition of the substrate, (ii) the extent of substrate contamination. This is influenced by the hygiene standards during production, equipment and ingredients contamination as well as the processing technology used and finally, (iii) the treatment of the products during storage and distribution [43].

Rapid Screening Techniques.

These are common techniques used in many developing countries including Ghana to control aflatoxin contamination. To ensure that only quality food material enters the food system, rapid detection methods and removal of contaminated seeds are employed. These techniques are either manual, mechanical or electronic and are used to remove damaged or discolored seeds from food stocks. These techniques have helped in many cases to control aflatoxin levels. For example, they have been used to reduce aflatoxin content to negligible levels in processed peanuts [30]. They mostly involve the use of low power microscopy [44], Blue Green Yellow (BGY) flourescence technique, hand sorting [45], air classification or bouyancy [46]. In Ghana, hand sorting and air classification are commonly used traditionally in the cereals and nuts production sector to ensure that quality food material enters the food system.

The disadvantages of these techniques are;

i. they have very low precision,

ii. high amount of waste due to loss in commodity and therefore it is not an economical process, finally

iii. it is not a very good preventive technique.

Environmental Control

Storage is one of the major areas of concern in terms of mold growth and aflatoxin production especially in most developing countries including Ghana. Therefore, prevention of mold growth and toxin production requires the need for good storage conditions. The environmental conditions prevailng in the storage facility play a significant role in the physicochemical and microbiological state of the commodity. The growth and proliferation of molds in most crops depend on a number of factors such as (i) water activity, (ii) storage temperature, (iii) state of the commodity, (iv) competing microbes and (v) the gaseous environment. These conditions influence the metabolism and the capacity of the molds to utilize the food material for growth and metabolite production [2].

Water activity (a_w)

Water activity is one of the most critical factors in the protection of stored products. It is the ratio of vapour pressure of the product to that of pure water at the same temperature and pressure. The lower the water activity the less water available for microbial growth and the inverse is a high moisture content. At low a_w, water is bound by salts, sugars, proteins, and other solutes, therefore growth of molds cannot occur since water is not present in an available form [47]. The minimum a_w for growth of *A. flavus* and *A. parasiticus* are 0.78 to 0.84 and 0.84 respectively, whilst that for aflatoxin production is 0.84 and 0.87 repectively [48]. The optimum a_w for aflatoxin production by both *A. flavus* and *A. parasiticus* is reported to be in the range of 0.95-0.99 [49]. Therefore, lowering water activity value of the food material to less than 0.78 inhibits the growth of and

becoming a rapidly emerging packaging technology of the future for the preservation of perishable and processed food products.

The novel method of active modification of the package atmosphere is through the use of oxygen absorbents and oxygen absorbent/CO_2 generators. These consist of sachets, like a dessicant, which are placed inside the packaged product and actively modifies the gaseous atmosphere. They are defined as "a range of compounds introduced into the MAP package (not product) to alter the atmosphere within the package" [66]. Various types are available to absorb only oxygen or to absorb oxygen and generate equal volumes of CO_2 within the package headspace. Oxygen absorbents in general act as a compliment to MAP, reducing oxygen level to approximately 0.0001% [67]. The capacity of these absorbers have definite boundaries, the efficiency and the length of control is determined to a large extent by the transmission rates of the packaging material and the rate of gas production by product [68]. Oxygen absorbers play a very significant role in that they produce an oxygen-free environment ensuring a longer shelf-life for foods by preventing growth of molds and aerobic bacteria, insect damage and oxidation of unsaturated fatty acids [69].

A similar concept involves the use of ethanol vapor generators which modify the gas atmosphere by the production of ethanol vapor within the package headspace [70]. However there is little or no data available on the application of this technique in the control of aflatoxigenic molds and the production of aflatoxin. Recent studies by Ellis (1993) [71] on the application of different types of oxygen absorbents (Ageless pack) and different films to control aflatoxin production, showed that mold growth was inhibited at ambient and sub-ambient storage temperatures with growth occuring at higher temperatures above 30°C. This was attributed to the saturation of the absorbent and therefore the loss in its absorbing capacity, as well as the continuous influx of oxygen across the barrier films which is enhanced at higher storage temperatures. Generally, the ability of these absorbents to scavenge oxygen depends on the free flow of oxygen around the absorbent and the permeability of the packaging film.

The most common and widely used method of active modification is gas packaging. This is simply an extension of the vacuum packaging technology. The technique involves packaging of product in an impermeable film, evacuation of air from the package, followed by the injection of the appropriate gas mixture and heat sealing of the package [72]. Gases commonly used are nitrogen, oxygen and carbon dioxide. Since these gases are those that we breathe, the gases used in MAP are neither toxic or dangerous, nor are they regarded as food additives. However, much studies relating to aflatoxin production has been in the area of CAS with very little being carried out in the area of MAP control of aflatoxin production. Recent studies by Ellis et al. (1993) [73] using Malt Extract Agar in a CO_2 enriched atmosphere with different concentrations of headspace O_2 (0-20%) showed that MAP was effective in controlling both growth and aflatoxin production by *A. flavus*. The results of the study showed that MAP was effective in controlling toxin levels at very low inoculum (10^1 spores/gm) at temperature abuse conditions, since increasing inoculum level under similar conditions resulted in higher aflatoxin levels [74]. These authors also observed that, the effectiveness of MAP in controlling aflatoxin production depends on the synergistic effects of other environmental factors namely water activity, pH, storage temperature and the headspace gas composition. This finding also conforms to the widely accepted fact that microorganisms show greatest tolerance to a single environmental factor, such as CO_2 concentration, storage temperature and pH when all other conditions are optimal for growth [75]. However, two or more simultaneous environmental conditions or "barriers" will be far more inhibitory than each barrier considered separately. This synergism between MAP and other environmental factors in effectively controlling aflatoxin production was also observed in studies on peanut using *A. flavus* [76].

The extent of growth of aflatoxigenic mold species under MAP conditions is influenced by the rate of O_2 consumption and CO_2 production and these metabolic processes depend on four major factors; the level of substrate water activity, the environmental storage temperature, the microbiological state of the substrate and the gas transmission properties of the packaging film. Each of these factors has a significant influence on growth, with a corresponding effect on aflatoxin production. These effects are shown in Figures 1 and 2 where changes in either substrate water activity, headspace oxygen (balance CO_2 : N_2) or storage temperature has a significant effect on the extent of mold growth (Log C.F.U), with a corresponding effect on aflatoxin production [76].

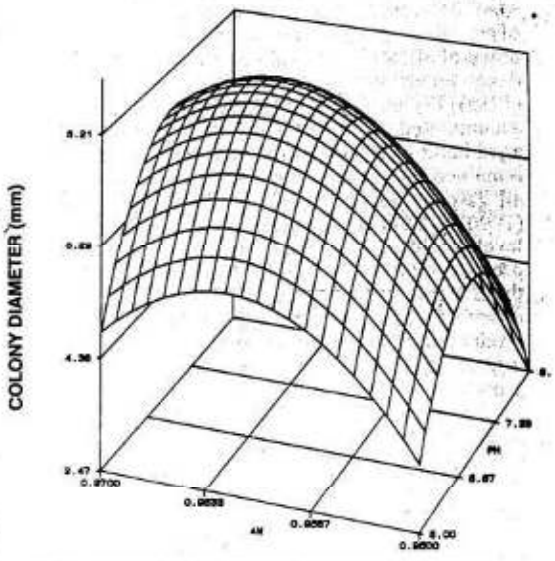

Fig. 1. Three dimensional Response Surface Graph showing the effect of water of activity (Aw) and pH on growth (mm) A *flavus* at 30 C and 5% O_2 concentration

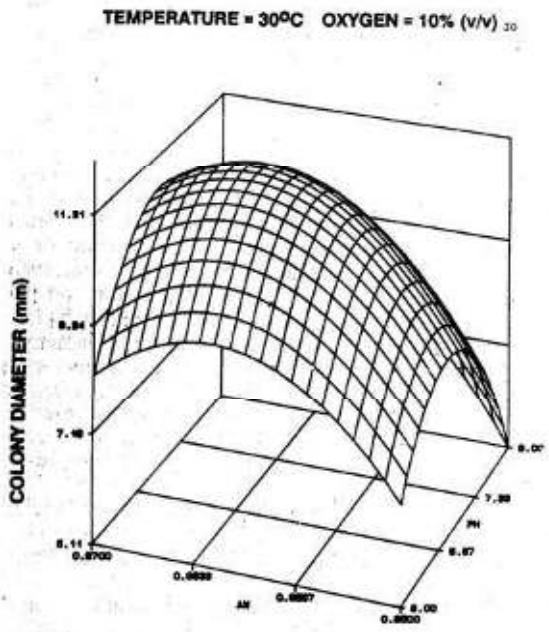

Fig. 2. Three dimensional Response Surface Graph showing the effect of water activity (Aw) and pH on growth (mm) of *A. flavus* at 30°C and 10% O_2 concentration.

Ellis et al. (1993) [76] also observed that with increasing storage time, the level of aflatoxin formed under MAP decreased. This was attributed to the depletion of available substrate for toxin production or the breakdown of aflatoxin as a substrate for further mycelial development and mold growth [18]. Buchanan et al. (1985) [77] found that aflatoxin production by *A. flavus* accumulated rapidly after 1-2 days in products packaged in air or 2% O_2 and then decreased rapidly and remained at a constant level (<2ug/mg dry weight) for all gaseous treatments. Karunaratne and Bullerman (1990) [78] also observed that at higher inoculum levels mold mycelia were capable of degrading the preformed aflatoxin at a faster rate than that at which the aflatoxins were formed. Ellis et al. (1993) [73] also observed a curvilinear relationship between aflatoxin production and growth with toxin production increasing to an optimum and then decreasing with increasing growth.

In the application of both CAS and MAP, CO_2 is primarily used as the biostatic agent. Four general mechanisms have been proposed for the preservative effects of CO_2 in foods;

(a) the displacement of oxygen by CO_2 thus inhibiting the growth of aerobic microorganisms, resulting in a delayed growth phase,

(b) the hydration of CO_2 to carbonic acid resulting in acidification of the tissues,

(c) either CO_2 or its ions may alter the bacterial cell permeability characteristics and

(d) the interferance of CO_2 in the metabolic pathways resulting in altered microbial enzymatic activity [79].

The biostatic or antimicrobial effect of CO_2 is also influenced by the packaging film permeability.

A good and efficient preventive method needs to satisfy the following criteria;

1. It should be simple,
2. Materials for control should be readily available,
3. Should be economical, i.e. minimal product loss and less expensive,
4. Should be non-toxic and environmentally friendly,
5. There should be minimal or no impairment to the nutritive value of the product and finally,
6. Treated or residual products should be suitable for both humans and animals .

Of the numerous control methods reviewed, many have been successful at the laboratory scale level. However, with the exception of ammonia, the implementation of most of these methods at the industrial scale level become difficult if the above principles are to be adhered to. Atmosphere modification inconjunction with other environmental factors seem to satisfy most or all of the above principles, with MAP and oxygen absorbers being of major advantage. Irrespective of the significant effect of MAP in the control of growth and aflatoxin production, its application in developing countries may be of concern except at the industrial scale level since the purchase of equipment, packaging materials and staff training would be an additional cost. However, this would be more economical compared to the CAS technique. This problem may be overcome by the application of the absorbent technology which does not require the use of expensive evacuation or gas flushing equipment. One limitation with the absorbent technology is consumer resistance to satchets in packaged products.

NEW AREAS FOR POTENTIAL RESEARCH

Recently, the use of natural anti-microbial agents such as chitosan have shown significant effect in controlling the growth and aflatoxin production by aflatoxigenic molds. Chitosan is the N- deacetylated form of chitin, which is non-toxic and biodegradable. It is easily obtained from the protective shells of crustaceans such as crabs and shrimps. It also forms the body-wall of

most fungi, molds and yeasts [80]. It has been shown that chitosan treatment reduced *A. flavus* occurrence and aflatoxin contamination in pre-harvest maize [81, 82]. These findings were also observed by Ellis et al. (1993) [83] in their studies on both *A. flavus* and *A. parasiticus* on Malt Extract media. Chitosan has also shown significant effect in inhibiting the growth of some bacterial and fungal species [84, 85]. Notwithstanding these observations, more studies need to be carried out especially with respect to aflatoxin, to determine the practical application of chitosan on agricultural products and its effect under different environmental conditions.

CONCLUSION

Aflatoxin contamination of food products continue to be of major concern worldwide, especially in tropical third world countries. Presently, chemical control programmes face many problems, such as the increasing amount of fungicide tolerant pathogens and the increasing resistance of the public to chemically treated food products. It is for these reasons that, there is the growing need to develop alternative approaches for the efficient control of aflatoxins taking into consideration cost and applicability, especially for developing countries. From this review, it can be concluded that the efficient control of aflatoxins in food products can be effectively achieved through a combination of methods. The use of oxygen absorbents and especially that of natural anti-microbial agents would be a very effective, economical and adaptable process for tropical developing countries. Therefore, further research needs to be carried out into the effective use of natural products such as chitosans for the control of aflatoxin production by aflatoxigenic molds.

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