FOOD AND NUTRITION NEWS

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Aflatoxins - its Prevention and Detoxification

There was a severe out break of Turkey 'X' disease in England in 1960s, which has killed about 1,00,000 turkeys and other farm animals. The cause of the disease was traced to a feed component, peanutmeal, which was infested heavily with Aspergillus flavus, which was discovered as a series of fluorescent compounds, later termed as aflatoxins. Aflatoxins are mycotoxins produced by molds, specially, *A. flavus* and *A parasiticus* which are ubiquitous in nature and all types of organic matter are suitable substrates for their growth.

Chemically aflatoxins are difurano coumarin derivatives, about 18 different types of aflatoxins have been identified with aflatoxin B_1, B_2, G_1, G_2, M_1 and M_2 being the most common and B_1 being most potent.

Fungi form part of indigenous microflora of air and soil and also frequent contaminants of food and agricultural commodities. The most important factors influencing growth of aflatoxin production by A. *flavus* and *A parasiticus* are relative humidity surrounding the substrate, which in most cases is between the 88 and 95% and storage temperature of 25C and 30C respectively. Groundnut and its products are most susceptible to aflatoxin contamination, however, other commodities such as maize, sorghum soyabean, cotton seeds, copra, oats, rice and wheat are also susceptible when stored with high moisture content.

Toxicity of aflatoxin : Aflatoxins are toxic to various domestic and laboratory animals, birds, plants and insects. The severity of toxic symptoms depends on the host species, their age, sex and nutritional status. In general, young animals are easier prey to aflatoxin than older ones and males are more susceptible than females. Thus toxic effects can be classified into 1) biochemical and 2) biological effects.

Bio-chemical effects: Aflatoxins may be considered as biosynthetic inhibitors both in vivo and invitro, affecting different metabolic systems. Aflatoxin B_1, G_1 and M_1 inhibit oxygen uptake in whole tissues by acting on electron transport chain system. They inhibit the activity of enzyme adenosine triphosphatase to varying degrees, resulting in decreased production of ATP. It has been reported by several investigators that hepatic glycogen levels are reduced by aflatoxin due to the inhibition of glycogenesis, depression of glucose transport into liver cells and acceleration of glycogenolysis.

Aflatoxin B_1 can be converted to it epoxide form which binds DNA, preventing transcription. Aflatoxin B_1 also binds with RNA inhibiting protein synthesis. Metabolities of aflatoxins can react readily with free amino groups of functional proteins, resulting in reduced enzyme activity.

Biological effects

Carcinogenesis : Aflatoxins are known to be potent carcinogens, causing cancer of the liver, colon and kidney in experimental animals. The toxicological effects of aflatoxin B_1 occur after the metabolic activation of molecule by the microsomal mixed function oxidase system, leading to the formation of highly reactive intermediates such as 2, 3-epoxy aflatoxin B_1. Binding of these reactive intermediates to DNA results in disruption of transcription, leading to abnormal cell proliferation.

Mutagenesis : The possible mechanism of mutagenesis is reported to be initiated by an aflatoxin B_1-DNA binding process, leading to the formation of single - stranded gaps as a result of DNA binding sites. This stimulates an error prone repair system that may induce mutation by 1) insertion of erroneous nucleotides opposite spontaneously recurring apurinic sites or (2) through errors during filling of single stranded gaps that do not contain additional DNA lesions.

Teratogenesity : Aflatoxin B_1 being a potent inhibitor of protein synthesis in eukaryotic cells, impairs differentiation of sensitive primordial cells. Susceptibility varies greatly on course of gestation and dosage of the toxin. A single dose of intraperitoneal injection of aflatoxin B_1 at 4 mg/kg body weight, administered to hamsters on 8th day of pregnancy caused a high proportion of malformed and dead or reabsorbed fetuses.

Hepatotoxicity : Reactive intermediates, such as 2,3 - epoxy aflatoxin B_1, react with macromolecules of the liver cells, resulting in fatty and pale livers, moderate to extensive necrosis and haemorrhage.

Prevention, and detoxification of aflatoxins in foods :

Prevention of mould contamination, producing aflatoxin can be achieved by several methods.

1. Improved farm management by preventing fungal attack at field level by adopting improved farm techniques.
2. Antifungal agents such as propionic acid and sorbic acid can effectively prevent the growth of aspergillus flavus and A. parasiticus.
3. Developing varieties which are resistant to mould attack or inhibit toxin production by genetic engineering.
4. Control of environmental conditions during storage to prevent the development of toxigenic strains of moulds such as moisture, temperature or modified gaseous atmosphere.

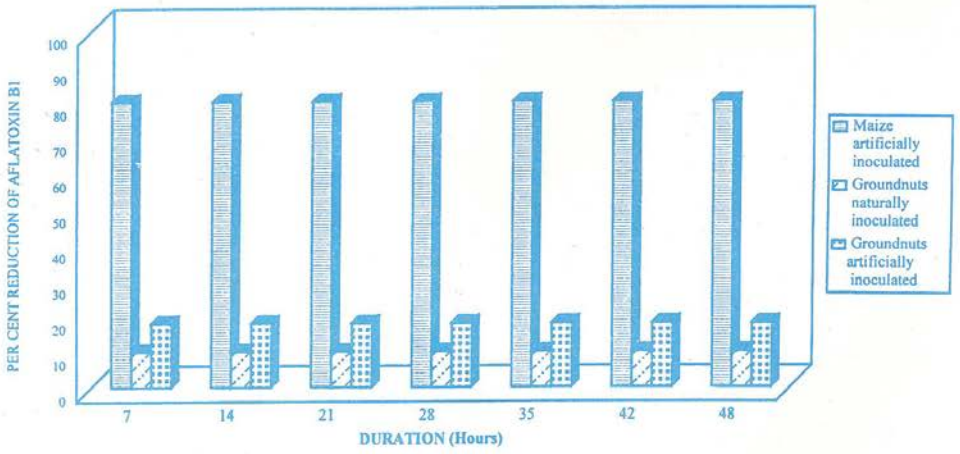

Fig. 1 : EFFECT OF SUNLIGHT ON PER CENT REDUCTION OF AFLATOXIN B1 IN MAIZE AND GROUNDNUT KERNELS

Preventing the contamination of food by the toxigenic fungi is the most rational and economic approach to avoid the potential hazards. However, prevention is not always possible under certain agroclimatic and storage practices. Therefore detoxification process has gained importance in salvaging food already contaminated.

Degradation treatments of aflatoxin B_1 are aimed either at removing the double bond of the terminal furan ring or in opening the lactone ring. Once the lactone ring is opened further reactions could occur to alter the binding properties of the terminal furan ring to DNA and proteins. Many physical and chemical methods have been developed to detoxify the aflatoxins. The work being carried out in this aspect at this institute is reported here.

Effect of sunlight on aflatoxin B_1 reduction in maize and groundnut kernels

Maize and groundnut kernels contaminated with aflatoxin exposed to sunlight for varied duration revealed that 80% reduction in aflatoxin B_1 has taken place in maize when exposed to sunlight for 7 hrs. No further reduction was observed after 7 hrs till 48 hrs. Similar treatment in groundnut has resulted in only 10.2-16.7% for naturally infected and artificially inoculated samples respectively (Fig.1) The reason for such larger differences in reduction of aflatoxin in these two grains was related to the photosensitivity which depends on the associated proteinaceous material and type of bonding with aflatoxins.

Effect of chemical treatment on aflatoxin production in maize and groundnut kernels.

Effect of sodium chloride, propionic acid, acetic acid at different concentrations and in combination on inhibition of aflatoxin B_1 production during storage upto 90 days was studied and the results are shown in Table 1. Treatment with

Table 1 : Effect of chemical treatments on aflatoxin reduction in stored maize and groundnut kernels.

Treatment	Concentration %	% reduction in aflatoxin B_1			
		Maize	Groundnut	Maize	Groundnut
1. Sodium Chloride	2.5	99.9	33.3	99.8	33.3
	5	100	99.5	99.5	99.5
	10	100	100	99.9	100
2. Propionic acid	1	99.9	100	100	100
	2.5	99.9	100	100	100
	5	99.9	100	100	100
3. Acetic acid	1	100	76.9	100	65.0
	2.5	100	100	100	100
	5	100	100	100	100
4. Combination of propionic & acetic acid and PA:AA (1:1)	1.0	100	100	100	100
	2.5	100	100	100	100
	5.0	100	100	100	100

Source : Waghray .K. and Uma Reddy M.

Table 2 : Effectiveness of different levels of clays on detoxification of aflatoxin B_1 in unrefined groundnut oil

Clay	level of clay (%)	Filtration time (Seconds)	Aflatoxin B1* (ng/g)	Percent reduction
1. Fuller's earth	3.0	150	ND	100.0
	1.5	90	ND	100.0
	1.0	75	16.67	83.3
	0.5	22	33.33	66.7
2. Kaolinite	3.0	420	ND	100
	2.5	300	4.17	95.8
	2.0	240	8.33	91.7
	1.5	80	12.50	87.5
	1.0	60	16.67	88.3
	0.5*	40	50.0	50.0
3. Ball clay Grade I	3.0	240	ND	100.0
	1.0	90	ND	100.0
	0.5	25	33.33	66.7
4. Ball clay Grade II	3.0	240	ND	100.0
	1.0	60	ND	100.0
	0.5	25	33.33	66.7
5. Kaolinite C	3.0	120	ND	100.0
	2.5	90	ND	100.0
	2.0	75	ND	100.0
	1.5	53	12.50	87.5
	1.0	30	16.67	83.3
	0.5	20	33.3	66.7
6. Kaolinite S	3.0	90	ND	100.0
	1.5	45	ND	100.0
	1.0	25	16.67	83.3
	0.5	20	33.33	66.7

* Initial level of aflatoxin B_1 in groundnut oil 100 ng/g, ND : Aflatoxin B_1 not detected.
Source : Waghray .K. and Uma Reddy .M. (1995)

all the three chemicals was found to be effective in inhibiting aflatoxin B_1 production completely upto 90 days at lower concentration of 1% level.

Detoxification of groundnut oil with selected aluminium silicate clays.

Effect of filtration of unrefined groundnut oil using selected clays at different temperatures and contact time on detoxification of aflatoxin B_1 was studied. Details of the study as shown in table 2 suggest that all the clays selected were effective at 3% levels in removing aflatoxin B_1 completely from the oil and Ball clay grade I and II were effective at 1% level. All other clays were also effective in reducing aflatoxin levels to permissible limits and at 1.5 - 3% levels removed the toxin completely. Filtration time has not shown any effect on the removal of the toxin from the oil.

Effect of different methods of processing on aflatoxin degradation in various food products :

Though aflatoxins are heat resistant, studies carried out at this institute have revealed that different methods of cooking which are normally carried out at home have significantly reduced the aflatoxin B_1 (Table 3). Boiling whole maize kernels, and on porridge making 51-58% of the toxin is degraded, roasting employed in roti making and in roasted groundnut the toxin reduction ranged from 60-75%. In fried product such as poori and muruku reduction was upto 85%. In baked products such as biscuits and muffin the toxin reduction was 67%. When two methods of processing are employed in preparation as fermentation and cooking, like idli, dosa and fermentation and baking such as bread, the reduction in aflatoxin B_1 was higher than in products where only single process is involved.

Table 3 : Degradationof aflatoxin in dif ferent products made with selected grains contaminated with aflatoxins.

	Treatment	Grains	Product	Aflatoxin B_1 degradation %
1.	Boiling 25 min	Maize	Boiled grain	51
2.	Boiling 8 min	Maize semolina	Porridge	58
3.	Boiling with salt, 15 min	Groundnut	boiled groundnuts	33
4.	Dry roasting 150°C 5 min.	Groundnut	Roasted groundnuts	75
5.	Roasting & boiling 3 min.	Wheat	Upma	67
6.	Pan baking, 3 min	Semolina maize flour	Roti	61.3
7.	Pan bakins, 3 min	Wheat flour.	Chapati	85
8.	Baking at 275°C,20 min	Maize flour	Biscuits	67.5
9.	Baking at 250°C, 10 min	Maize flour	Muffins	61.1
10.	Fermentation for 18/5 andskanins 10 min	Maize flour Black gram dhal	Idli	70
11.	Fermentation 14 hr + steaming 10 min	Rice and black-gram dhal	Idli	86
12.	Fermentation 14 hrs + shallow frying 2 min	Rice and Black-gram dhal	Dosa	86
13.	Fermentation & baking 200°C, 25 min	Maida	Bread	80
14.	Frying in oil, 150°C for 30 seconds.	Wheat flour	Poori	85
15.	Frying in oil, 150°C for 4 min.	Rice flour and bengalgram flour	Muruku	78
16.	Frying in oil 150°C 2 min.	Groundnut.	Fried groundnuts	73

Source : Shenoy. S and Uma Reddy and Pratima Rani and Uma Reddy (under publication)

Dietary Fibre and Resistant Starch Contents of Millets on Processing

Preparation of food grains for consumption involves processing by both primary and secondary methods. The processing methods applied especially those involving heat result in a chain of interactions between several constituents of the food which affect the content and bio availability of certain nutrients.

Cereal and millet grains are major sources of starch and also contain dietary fibre which constitutes the unavailable carbohydrate component. It has been reported that dietary fibre (DF) content of cereal/millets can be increased without using any additives but by heat processing the grains. This is due to a part of the available carbohydrates which becomes unavailable on processing and thus resists enzyme hydrolysis in the small intestine. This fraction of starch designated as resistant starch (RS) has been considered to be the cause for increase in dietary fibre in processed foods based on foodgrains.

In a study undertaken at this institute, sorghum and pearl millet were subjected to traditional processing methods commonly employed in semi-arid regions of India. There was an increase in total dietary fibre (TDF) and resistant starch (RS) contents in all heat processed products. (Table 4). Starch from processed millet samples was easier to degrade than that from unprocessed grains. Higher starch digestibility was observed on boiling and baking because

there was an increase in soluble dietary fibre fraction.

Decrease in protein digestibility especially in sorghum products indicates complexing of millet proteins and inhibition of protein utilization by RS.

Factors which were identified to be involved in RS formation were moisture content in food preparation, degree of starch gelatinisation, amylose content and time of heat contact.

The area of study on RS is fairly new and is of great importance for those population groups deriving most of the energy and protein from these grains because change in dietary fibre affects availability of starch and protein.

Kamini Devi
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Table 4 : Mean TDF, RS, IVSD and IVPD values in raw and processed millets.

Grain & Product	TDF (%)	RS (%)	IVSD (%)	IVPD (%)
Sorghum				
Milled, raw	10.3	0.51	21.2	65.6
Dehulled, raw	8.6	0.52	23.0	63.6
Germinated	10.8	0.76	26.8	69.7
Dehulled, boiled	11.7	1.17	59.3	31.2
Popped	11.2	0.96	54.3	36.5
Baked (roti)	10.9	1.04	58.3	29.9
Pearlmillet				
Milled, raw	9.4	0.70	21.3	78.0
Dehulled, raw	8.5	0.70	24.4	74.5
Germinated	10.5	0.65	27.8	82.5
Dehulled, boiled	8.9	1.49	59.1	56.2
Popped	9.8	0.82	44.0	40.4
Baked (roti)	10.5	1.39	63.5	35.1

TDF : Total dietary fibre IVSD : Invitro starch digestibility RS : Resistant Starch IVPD : Invitro protein digestibility

Valedictory function of short course on "Assessment of Nutrition Status" Organised by Centre of Advanced Studies, Department of Foods and Nutrition

Short course on "Assessment of Nutritional Status" was organised from 8th to 27th July 1996 at P.G. & Research Centre, A.P. Agricultural University, Hyderabad. The course was co-ordinated by Dr. M. Uma Reddy and Dr. P. Yasoda Devi.

Eleven inservice teachers from various universities have participated in the course. After completion of the course, valedictory function was organised on 27th July 1996. The chief guest of the function was Dr. M. Mohan Ram, Director, National Institute of Nutrition and was presided by Dr.M.V. Rao, Vice Chancellor, A.P. Agricultural University. Dr. Sugunakar Reddy, Dean of Home Science gave the introductory remarks. The gathering was welcomed by the Principal, Dr. S. Renuka. Dr. Vijaya Khader, Director, Centre of Advanced Studies explained the objectives of Centre of Advanced Studies. Dr. M. Uma Reddy, Course Co-ordinator gave the report on the short course, followed by the participants remarks. Dr. Mohan Ram in his address stressed the need for short courses to update the knowledge in the subject. He also enlightened the gathering on the importance of Nunitional status assessment and its

relevance to operate the nutritional programmes. Later he distributed the certificates to the participants. Dr. M.V. Rao in his presidential remarks explained the efforts made by the faculty members in getting the Centre of Advanced Studies and appealed to the staff to work hard to maintain the academic standards in teaching to achieve number one position in the country. Dr. R. Manorama proposed vote of thanks.

AWARDS

Dr. M. Uma Reddy, Associate Professor in Foods and Nutrition Department, has been awarded G.S. Nivetia Memorial Award 1995, for the best Research Paper published in all issues of the Journal of the Oil Technologists' Association of India in 1995. The Research Paper was on "A simple method for decontamination of Aflatoxin B1 in unrefined groundnut-oil using aluminium silicate clays, co-authored by Dr. Kavita Waghray.

The award includes citation and a cash prize of Rs. 5,000/-

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