NARO

MANAGEMENT OF AFLATOXINS IN GROUNDNUTS

A manual for Farmers, Processors, Traders and Consumers in Uganda

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NATIONAL SEMI-ARID RESOURCES RESEARCH INSTITUTE
NATIONAL AGRICULTURAL RESEARCH ORGANISATION

2010

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Citation: Okello, D. K., Kaaya, A. N., Bisikwa, J., Were, M., & Oloka, H. K. 2010. Management of Aflatoxins in Groundnuts: A manual for Farmers, Processors, Traders and Consumers in Uganda. National Agricultural Research Organisation, Entebbe.

ISBN: 978-9970-401-00-0

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Foreword

Aflatoxins are not visible neither do they have a particular flavour. Therefore, it is not easy to convince consumers about their existence in food. The majority of farmers, traders and consumers in Uganda are not currently aware of the aflatoxin contamination of food and feed and their implications on commerce, human and livestock health. Aflatoxin contamination can occur in the field, during postharvest drying and storage, and shipment. Crop husbandry practices, climatic conditions, and soil factors, in addition to host-plant susceptibility, significantly influence aflatoxin contamination.

Groundnut is one of the most susceptible crops to aflatoxin contamination yet it is a major staple, widely cultivated and consumed in Uganda. Raising awareness of aflatoxins and disseminating relevant information to individuals is therefore an important part of any intervention strategy to manage the problem. Increased awareness will help enhance the adoption of the technologies available to minimize aflatoxin contamination. Various information pathways can be used to increase awareness about the dangers of aflatoxins in food and feed. These include development and distribution of manuals, flyers and brochures in major languages, a farmer-participatory approach to technology evaluation and dissemination, conducting training courses and workshops, and use of media e.g. radios, televisions and newspapers. Awareness campaigns should utilize already existing systems for disseminating information to subsistence farmers. Such campaigns should also include the dissemination of information to non-governmental organizations, public service institutions, agriculture extension agents, health care providers, and schools. Given diversity in culture and remote location of villages, multiple means for disseminating information as part of an awareness campaign may be necessary to reach a broad range of people.

To strengthen the local capacity to monitor aflatoxin contamination in Uganda, infrastructure for aflatoxin diagnostics needs to be established in major groundnut growing areas and trade points; and local personnel need to be trained to manage these facilities. Strengthening the local capacity to monitor aflatoxins is necessary for developing countries like Uganda to regain the high-value export groundnut trade. Such monitoring requires the availability of cheap, accurate and rapid-testing procedures, such as ELISA-based methods or other technologies that match location-specific needs with the socio-economic profiles of farmers in developing countries. Regulations for monitoring susceptible produce from farm level through buying points to retail markets should be put in place and strict measures on food quality at both household and market levels should be embraced and enforced by all policy makers.

At farm level there are many factors that influence the aflatoxin contamination of peanuts most of which are environmentally related, such as weather and insects, and these are difficult, if not impossible, to control. Particular attention should be paid to the soil population of the fungus, the health of seed material, soil moisture deficit stress at the pod formation and pod maturity stages, and rains at harvest. The critical control points often do not exist at the pre-harvest level. However, after harvesting, these critical control points may be identified for aflatoxins produced by fungi during drying and storage. For example, a critical control point could be at the end of the drying process and one critical limit would be the moisture content/water activity.

It is recommended that resources be directed to emphasizing the Good Agricultural Practices (GAPs) at the pre-harvest level and during drying and storage and Good Manufacturing Practices (GMPs) during the processing and distribution of various products.

The implementation of the guidelines contained in this manual will minimize aflatoxin contamination of groundnuts through applications of preventive control to the extent feasible in the production, handling, storage and processing of each groundnut crop.

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Contents

Tables

Figures

Acknowledgements

The National Agricultural Research Organisation and the authors greatly acknowledge support from the government of Uganda, through the Ministry of Agriculture, Animal Industry, and Fisheries that supported the publication of this work. Credit also goes to the Carnegie Foundation that supported research that enriched this manual through a grant (Food, Nutrition and Value Addition Phase III) to Makerere University. The Authors particularly thank the management of National Semi-Arid Resources Research Institute for support and encouragement during preparation of this document.

Acronyms

DNA	deoxyribonucleic acid
EU	European Union
FAO	Food and Agricultural Organisation of the United Nations
FDA	Food and Drug Administration of the United States of America
HIV/AIDS	Human Immunodeficiency Virus / Acquired Immune Deficiency Syndrome
HPLC	High performance liquid chromatography
HPTLC	High performance thin-layer chromatography
MAK	Makerere University
NARO	National Agricultural Research Organisation
NaSARRI	National Semi-Arid Resources Research Institute
ppb	parts per billion
RNA	ribonucleic acid
TLC	Thin layer chromatography
UNBS	Uganda National Bureau of Standards
UV light	Ultra-violet light
WHO	World Health Organization of the United Nations

Chapter 1: Introduction

1.1 Groundnuts in Uganda

Groundnut *(Arachis hypogaea* L.), also known as peanut, is the second most important legume after beans *(Phaseolus vulgaris L.)* grown mainly in Eastern and Northern Uganda but consumed widely throughout the country (Okello *et al.*, 2010). There has been a substantial increase in groundnut production as both a food and cash crop because of increased awareness of their value as a source of protein (23-25% content), fat (40-50%), oil (40-52% content), and 10-20 % carbohydrate depending on the variety (Savage and Keenan, 1994). With the costs of animal protein ever increasing, groundnut is becoming an even more important source of protein. A kilogram of groundnuts is high in food energy and provides approximately the same energy value as 2 kilograms of beef, 4 litres of milk, or 36 medium-size eggs. Groundnut seeds are also a nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium. Groundnut is consumed raw, roasted, blanched, as peanut butter, crushed and mixed with traditional dishes as a sauce or as *binyebwa*, a cooked paste. These qualities make groundnut an important nutritional supplement to mainly cereal diets of maize, millet and sorghum of many Ugandans.

Groundnuts thrive under low rainfall and as a legume, groundnuts improve soil fertility by fixing nitrogen. The crop generally requires few inputs, making it appropriate for cultivation in low-input agriculture by smallholding farmers. In Uganda like many sub-Saharan African (SSA) countries, women predominantly grow and manage the crop. Therefore, groundnut cultivation has a direct bearing on the overall economic and financial well-being and nutritional status of women and children (Okello *et al.*, 2010). As a cash crop it gives relatively high returns for limited land area, and is well adapted to the hot, semi-arid conditions of Uganda. These multiple uses of groundnut make it an excellent staple food and cash crop for domestic markets as well as for foreign trade. Groundnut production, marketing and trade provide sources of employment, income and foreign exchange. However, lack of cottage technologies for value addition and product development as well as the mechanisms for controlling mycotoxins especially Aflatoxin have impacted negatively on the realization of the full economic benefit from groundnut production in the region (Kaaya *et al.*, 2006). The unacceptable levels of aflatoxin in groundnut have the potential to limit its commerce and use in human and animal diets.

1.2 Mycotoxin problem in grains

Several species of fungi infect agricultural crops both in the field and during storage. These include *Aspergillus, Fusarium, Penicillium, Alternaria, Cladosporium* and *Nigrospora* species (Hocking, 1991), and have been mainly found associated with cereals, nuts and spices. In addition to reduction of yield to these crops, some of these moulds produce mycotoxins.

Mycotoxins are toxic substances produced by fungi and can be classified according to their fungal origin, chemical structure and biological activity (Smith and Moss, 1985). Occurrence of these toxins in human foods is mainly as a result of direct contamination of agricultural commodity and their survival of food processing to some extent (Scott, 1991).

Oilseeds especially groundnuts (peanuts), maize (corn), soybean, cottonseed, and copra are particularly favourable substrates for mycotoxin formation. Diseases in animals and human beings resulting from consumption of mycotoxins are called mycotoxicoses. Because of their serious effects, the incidence of moulds and levels of mycotoxins in foods and feeds should be frequently and routinely monitored.

Over 200 mycotoxins have been reported but only those occurring naturally in foods are of significance in terms of food safety. These are produced mainly by species of *Aspergillus*, *Penicillium* and *Fusarium* (Table 1).

Table 1: Common mycotoxins found in foodstuff

Mycotoxin	Main causal agent	Foods commonly contaminated
Aflatoxin	*Aspergillus flavus, A. parasiticus*	All grains, dried fruits
Fumonisin	*Fusarium verticillioides*	Maize
Zearalenone	*Fusarium graminearum*	Maize
Ochratoxin	*Aspergillus ochraceous*	Coffee, cocoa
Trichothecenes (T2 Toxins and deoxynivalenol)	*Fusarium spp*	Cereals (wheat, barley, maize, rice)
Patulin	*Penicillium digitatum*	Apples

The most widely studied and dangerous mycotoxins are aflatoxins (Kaaya and Warren, 2005). *A. flavus* (Fig. 1) is common and widespread in nature and is most often found when certain grain is grown under stressful conditions such as drought. This mould, according to Miller (1991) is found widely on inadequately dried food and feed grain in subtropical and tropical climates throughout the world.

There are four major aflatoxins: B_1, B_2, G_1, G_2 plus two additional metabolic products, M_1 and M_2 that are of significance as direct contaminants of grains (FAO, 2002). The aflatoxins M_1 and M_2 were first isolated from milk of lactating animals fed aflatoxin contaminated rations; hence, the M designation. The B designation of aflatoxin B_1 and B_2 resulted from the exhibition of blue fluorescence under UV-light, while the G designation refers to the yellow-green fluorescence of the relevant structures under UV-light. The chemical structures of these toxins are presented in Fig. 2.

It should be noted that it is difficult to eliminate aflatoxins completely from food after they have developed, although some reduction can occur during processing. Aflatoxins persist under extreme environmental conditions and are even relatively heat stable at temperatures above 100°C, the boiling point of water (Jacobsen *et al*. 1993).

Despite the importance of groundnuts as food and feed, the presence of aflatoxins has the potential to limit its use especially in human diet. Aflatoxin contamination is considered as one of the most important groundnut quality problems in Uganda (Kaaya *et al*., 2006). Mycotoxigenic fungi and aflatoxin contamination in groundnuts starts at farm level and contamination occurs in both pre and postharvest phases.

The pioneering effort in the survey of aflatoxin content of foods and food products in Uganda was undertaken in early 1966 (Lopez and Crawford, 1967). The content of aflatoxin was estimated in groundnuts sold for human consumption in the country. About 15% of the samples examined contained more than 1 ppb of aflatoxin B_1 and three percent contained more than 10 ppb. Studies conducted in 2000 in Kumi and Mayuge districts established that 48% of the groundnuts in Kumi stored by farmers for up to seven months and 28% of those newly harvested tested positive for aflatoxins, with ranges of 0 – 22 ppb and 0 - 5 ppb, respectively (Kaaya *et al*., 2000). In Mayuge, 50% of the groundnuts stored for up to five months were positive for aflatoxins, with a range of 0 – 18 ppb.

Another study conducted in 2003-2004 (Kaaya *et al*., 2006) to determine the aflatoxin content of groundnuts from farms and markets (wholesalers and retailers) in Mayuge, Iganga and Mubende districts of Uganda and from St. Balikuddembe, Nakawa and Kalerwe – the three busiest markets in Kampala – indicated aflatoxin levels increased along the chain up to retail markets. All forms of groundnuts obtained from retailers in markets had levels of aflatoxin significantly higher than the recommended 20 ppb by the WHO/FDA.

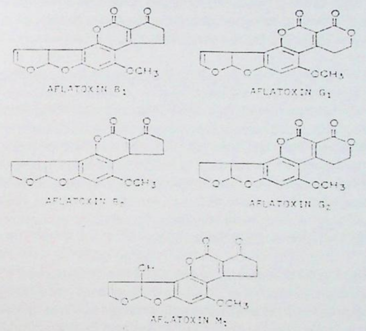

Figure 2: Chemical structures of aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin B2, aflatoxin G2, and aflatoxin M1. (Source: Palmgren and Hayes, 1987)

1.3 Factors promoting aflatoxin contamination of grains

Aflatoxin contamination of foods and feeds highly depends on biological (biotic) and environmental (abiotic) factors that lead to mould growth and toxin production and can occur both pre- and post-harvest. For example, mechanical damage, insect and bird damage, drought, stress and excessive rainfall encourage preharvest mould growth and aflatoxin production (Miller, 1991). Strain variation in the fungus, interference by other micro-

organisms, moisture, temperature, pH, the gaseous environment and preservatives are also important factors. In addition, the incidence and levels of fungal infection and aflatoxin contamination reported vary markedly from one geographical area to another (Smith and Moss, 1985; Kaaya *et al*., 2006). In most instances, however, aflatoxins are formed after harvest, particularly when harvesting takes place during floods, or unseasonal rains or when there is improper storage of insufficiently dried agricultural commodities.
The following factors have been singled out as those that mainly encourage mould growth and aflatoxin production in grains and kernels:

Moisture content: The amount of moisture in a grain affects both grade and storability and has a critical effect on mould growth and mycotoxin production. It is one of the most important considerations in determining whether aflatoxin will develop in groundnuts after harvest. Storage fungi grow at moisture contents in equilibrium with relative humidities ranging from 65-70 to 85-90 percent. *A. flavus* will only grow when the moisture content exceeds 9%, at 80-85% relative humidity and above.

Soil moisture stress has also been reported to enhance pre-harvest aflatoxin contamination of produce. Groundnuts exposed to drought stress in the field have been reported to have more *A. flavus* infected kernels than in irrigated plots. Excessive drought causes strains on pods and testas thus providing entry points for infection by fungi while excessive moisture weakens the pods and testas causing the same effect.

Temperature: The effect of temperature is difficult to separate from the effect of moisture. Under favourable temperature and relative humidity conditions, aflatoxigenic fungi grow on certain foodstuffs, most commonly grain like cereals and nuts. Production of aflatoxins is optimal at relatively high temperatures, so contamination is most acute and widespread in warm, humid climates. Under tropical conditions typical of Uganda, stored products are more susceptible to *Aspergillus* species than other fungi, as many *Aspergilli* are favoured by the combination of low water activity and relatively high storage temperatures (Pitt and Hocking, 1997). *Aspergillus flavus* grows best between 10°C and 45°C at a relative humidity of 75% or more although the optimum conditions for aflatoxin production are between 25°C and 30°C, at 85% relative humidity (FAO, 1998).

Handling and drying: Mechanical damage to kernels makes them much more vulnerable to invasion by storage moulds, including *A. flavus*. Under any given environmental conditions fungal growth is several times faster in damaged compared to intact kernels. Cracks and breaks in grains are caused mainly during harvesting and shelling, although insect and rodent feeding may also be responsible for breaks in the pericarp (Sauer and Tuite, 1987).

Traditional groundnut drying techniques in developing countries like Uganda involving field and bare ground drying are a major source of fungal contamination. They are slow, time consuming and labour intensive involving lots of crop handling (that the resource limited farmer may not adequately accomplish), and due to rains that normally persist at harvesting and drying times, it is difficult to achieve the recommended moisture level for safe storage. In addition, the crop is persistently exposed to soil contamination which is the source of fungi (Kaaya *et al*., 2007; Okello *et al*., 2010).

Storage Conditions: The fundamental reason why commodities are stored dry is to increase storability and in part, prevent growth of storage fungi. If commodities are incorrectly stored, that is, in an improperly dried state or under high humidity with inadequate protection, fungi will inevitably grow. Duration of storage is an important factor when considering mycotoxin formation. The longer the retention in storage the greater will be the possibility of building up environmental conditions conducive to aflatoxigenic mould proliferation in groundnuts (Kaaya *et al*., 2000).

Storage structures commonly used by farmers in Uganda are traditional and may not maintain an even, cool and dry internal atmosphere; they do not provide adequate protection from insects and rodents; are not easy to clean and above all, are not water proof. All these conditions favour mould growth and aflatoxin production.

Insect infestation or damage: Insect infestation during storage is one of the major problems facing farmers in Uganda. Insects and mites may damage stored grain, but they also interact with fungal colonisation in many different ways. Fungal spores can be carried by insects. Toxin-producing fungi can infect growing crops, as a consequence of insect damage and may produce toxins prior to harvest or during harvesting and storage. During storage, insects, due to their metabolic heat and water, can increase the water activity and temperature of grain to levels suitable for fungal growth. Thus, it is important that insects are controlled both pre and postharvest (Hell, 2000).

1.4 Nutrition and health effects of aflatoxins

Aflatoxin (especially aflatoxin B1) recognition as potent carcinogens in animals and humans has made them subjects of government legislation as well as valuable tools in the study of cancer. There are a range of possible consequences of exposure to aflatoxins, largely determined by the dose, the duration of exposure, and the animal involved. In all cases, the young of species are much more susceptible than the adults, and nutrition can be an important factor.

Acute illness and death: Acute illness is as a result of consumption of foods contaminated with very high levels of aflatoxin. Individuals die as a result of jaundice and liver failure. In 2004, more than 200 people died in Kenya and more children have died this year (2010) as a result of consuming maize contaminated with aflatoxins (*Media reports*). No animal species is resistant to acute toxic effects of aflatoxins (Williams *et al*., 2004)

Chronic illnesses/ Cancers: The International Cancer Research Institute identifies aflatoxin as a Class 1 carcinogen. This classification is the basis for the regulation of this toxin to exceptionally low levels in traded commodities (US 10 ppb in grain; and 0 ppb in milk; EU 4 ppb and 0 ppb in milk).

Aflatoxin is predominantly perceived as being associated with liver cancers. The metabolites, especially those of aflatoxin B_1 are capable of binding to protein, DNA and RNA thus interfering with the normal cellular functions resulting in initiation of carcinogenesis, mutagenesis or necrosis of the liver. For developing countries, the synergistic effects of aflatoxin compound the risk due to Hepatitis B virus (HBV), which is the other predominant cause of liver cancer.

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Immunology: Aflatoxins have been reported to reduce immunity in humans and animals. This is as a result of their interference with activities of important cells that boost immunity in the body. Thus, aflatoxins have been strongly linked to HIV/AIDS and malaria in Africa.

Nutritional illnesses: In animals it is established that aflatoxin in the diet decreases the rate of growth and other measures of productivity. In children especially those below three years aflatoxin exposure enhances stunting and underweight. Aflatoxins have also been implicated in the slowed rate of recovery from protein malnutrition (kwashiorkor). Generally, from the animal health perspective, aflatoxins cause growth reduction due to protein synthesis interference and micronutrient (vitamins A, B_{12}, C, D and E; minerals zinc, selenium, iron and calcium). This could be the reason why these toxins have been related to several nutritional-related illnesses in humans. Therefore, contamination of produce by aflatoxins puts consumers at high-risk health hazards.

1.5 Economic effects of aflatoxins

Aflatoxins in groundnuts, and indeed in all crops, can have direct economic effects resulting in loss of produce or loss of market value as well as indirect economic effects from loss of animals, increased costs of veterinary and human health care services, costs for food-borne disease surveillance and food monitoring. Presence of high levels of aflatoxins in groundnuts may make it unacceptable for marketing, causing financial loss to the farmer or retailer.

Depending on the market, economic losses may reach 100%, when the entire produce/ product is rejected by the market if aflatoxin levels are higher than acceptable standards. It is estimated that Africa loses over United States dollars 670 million annually due to requirements for European Union aflatoxin standards for all food exports and world over, billions of dollars are lost by farmers and traders due to aflatoxin contamination (Otsuki *et al.*, 2001; Guo *et al.*, 2009). It is therefore, very essential that all parties involved in the process of producing and marketing groundnuts should ensure that contamination from mycotoxins is minimized as much as possible (Table 2).

Aflatoxins have been known to be highly carcinogenic, and recent evidence from Uganda (Kitya *et al.*, 2010) have further reinforced the concerns over aflatoxins. Disease burden on farmers and the citizenry pose direct economic costs to persons and governments concerned.

Table 2: Examples of types of economic losses associated with aflatoxin (and other mycotoxins) contamination

Bearer	Economic losses and costs
National level	
Primary producer	• Outright food and feed loss • Less income from contaminated food • Reduced productivity of livestock
Intermediary	• Less income form products refused, condemned or sold at a discount • Increased storage, transport, and packing costs • Potential loss of market • Increased costs due to surveillance and control
National government	• Lower forex from reduced exports • Increased costs due to surveillance and control • Increased costs of shipment, sampling and analysis of products for export • Increased need for expenditures in human health and livestock care services • Increased costs of training, communication and extension programs
Consumer (human or livestock)	• Impaired health and productive capacity • Possible higher medical and veterinary costs
International level	• Loss of market value or market • Trade distortions

(Adapted from Jemmali, 1987).

1.6 Objectives of this manual

This manual has been developed to:

- To provide guidance on best practices in limiting aflatoxin contamination in groundnuts to acceptable levels
- To contribute to healthy living, among groundnut producing and consuming communities through utilisation of high quality, aflatoxin free groundnut and groundnut products, and
- To increase economic value of groundnuts and its products.

1.7 Scope

This manual has been prepared as a guide to farmers, processors, traders, and consumers of groundnuts in Uganda. It is meant to provide easy to use and best practices steps in the management of fungal toxins (especially aflatoxins) in groundnuts under Ugandan farming, climatic, and market conditions. The manual does not apply to other grains as handling practices and grain attributes tend to differ among crops.

Chapter 2: Safety standards, policy and testing for aflatoxins in groundnuts

2.1 Maximum tolerable levels and enforcement

Uganda does not have clearly set standards on aflatoxin contamination based on aflatoxin levels in most local foodstuffs. The Uganda National Bureau for Standards (UNBS)) in collaboration with other bureau of standards from the East African Community has nonetheless set a limit of 10 ppb for all foods and feeds but only currently certifies products intended for export. Other countries have different maximum tolerable levels of aflatoxin contamination with the EU having the most stringent standards (Table 3).

Table 3: Maximum level of total aflatoxin in foodstuffs

Country	Product	Maximum tolerable limit (ppb)
EU[1]	Groundnuts – Ready to eat	4
	Groundnuts – for further processing	15
USA	Groundnuts (all products)	20
India	Groundnuts (all products)	30
Kenya	Groundnuts (all products)	10
Uganda	Groundnuts (all products)	10

In Uganda, UNBS is the government agency charged with ensuring that all products are safe for consumption. Enforcement of maximum tolerable levels of aflatoxins would however be a very challenging process as most groundnut products in the country are traded informally. Peanut butter, flour, roasted nuts, and grain are mostly sold unpackaged or in inadequate packaging implying that enforcement of any maximum acceptable levels standards would prove very complicated. The UNBS however recognizes this problem and is already in the process of securing laboratory and human capacity to test levels of aflatoxin contamination in foodstuffs.

2.2 Effectiveness and gaps in policy

There is currently no central policy or strategies on aflatoxin contamination and management in Uganda. However, capacity exists within the country to test for aflatoxin levels in foodstuffs. A number of research efforts have been commissioned to develop control strategies for aflatoxins in groundnuts and other affected grains. There is need for clear procedures for aflatoxin control, monitoring and supervision in the country.

[1] EU, 2010

Figure 1: *Aspergillus flavus* growth on groundnuts in a petri dish

Figure 3: Groundnuts heaped in the house after harvesting: Bad practice

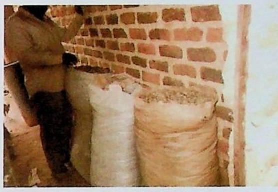

Figure 4: Soiled fresh groundnuts stored in bags prior to drying: Bad practice

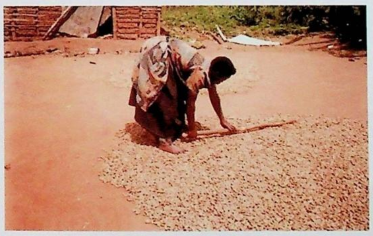

Figure 5a: Drying of groundnuts on bare ground, bad practice

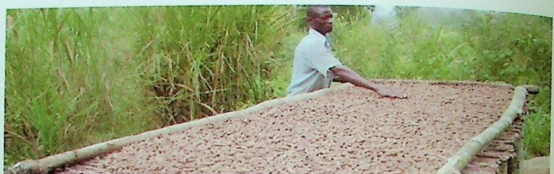

Figure 6: Drying groundnuts on a rack constructed from local materials - Recommended

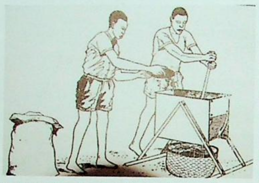

Figure 7a: Hand operated groundnut sheller commonly used in Uganda

Figure 7b: Motorised sheller used in Uganda

Figure 7c: Another Motorised sheller in Uganda

Figure 8: Groundnut kernels (sound and broken) in a bag delivered to the market in Kampala.

Figure 9: Sound kernels (in the metallic container) and broken kernels (spread on plastic sheet) during sorting of mixed grains from a plastic bag

Figure 10: A woman opening up a granary to get food

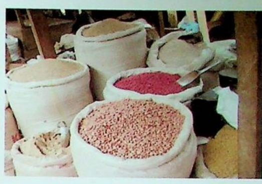

Figure 11: Storage of groundnuts at a retail market in Kampala

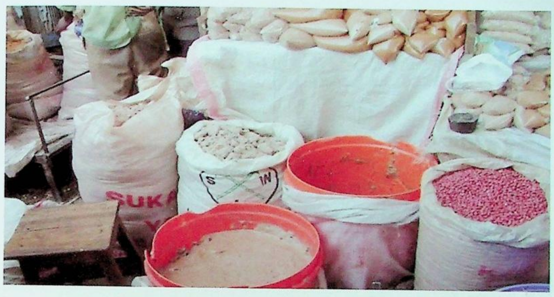

Figure 12: Groundnuts products commonly sold in retail shops in Uganda

Figure 13: a) Imported peanut butter

Figure 13: b) Locally made peanut butter

The problem will require a holistic approach that should be implemented by various government agencies as well self regulation by traders, processors and consumers. The ministry responsible for agriculture, should, for instance encourage farmers to use harvesting and storage techniques that limit fungal growth; the ministry responsible for trade, through the standard agency (UNBS) could certify all products and conduct inspections for products in shelves to ensure that mycotoxin contamination is at acceptable levels; while the ministry responsible for health, that currently houses the National Food and Nutrition Policy, should, in partnership with other institutions promote awareness campaigns on the dangers posed by aflatoxins so that consumers become aware of the need to handle groundnut products appropriately.

A fully national policy on aflatoxin contamination, together with enforcing regulations and organs is necessary, as is the case with many other countries in Africa, Asia, Europe and the Americas.

2.3 Aflatoxin analysis in groundnuts and groundnut products

In Uganda, testing for aflatoxin content can be conducted at Makerere University's Department of Food Science and Technology and at UNBS laboratories. Any person interested in performing such analyses is advised to consult the two authorities for assistance.

Sampling: Sampling is an important step in testing groundnuts for aflatoxin contamination. If aflatoxins were homogeneously distributed throughout the samples intended for analysis, then sampling should not have posed any problem. Unfortunately, the highly skewed nature of the distribution of aflatoxin complicates the sampling procedure. Toxicity often resides in only a few contaminated kernels. Additionally, there is extreme variation in the level of aflatoxin among contaminated kernels.

Two kinds of risks are recognized in testing for aflatoxins, the producers' risk and the consumers' risk. The consumers risk is an assessment of the likelihood of accepting a batch of groundnuts whose aflatoxin content exceeds the maximum level imposed for the toxin and the producers risk is an assessment of likelihood of rejection of a batch that contains less toxin than the statutory maximum level. Increasing the sample size will reduce both the consumers' risk and the producers' risk.

Sample preparation: Proper grinding and subdivision of the sample is essential before performing analysis for aflatoxins. Ideally, a sub sampling mill should simultaneously grind and subdivide the sample, and that developed by Dickens and Salterwhite is suitable for this purpose. Alternatively, the sample may be ground and subdivided in separate operations. The size of the sub sample may vary, but for most cases it ranges from 20 to 100 gm. A sub sample size of 50 gm is used in most methods and appears to be the best for obtaining both solvent economy and representative sample.

Methods for analysis of aflatoxins: Three types of assays have been developed for the detection of aflatoxins. These include biological, chemical and immunochemical. Biological assays were used when chemical and immunochemical methods were not available for routine analysis. Biological assays are qualitative or at best semi-quantitative, and often are non-specific. They are time consuming.

Chemical and immunological assays are suitable for routine analysis. They are invariably quick, and are cheaper and more specific than biological assays. Additionally, these tests are reproducible and sensitive. Chemical tests available for aflatoxin analysis are thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high performance liquid chromatography (HPLC) and fluorometric methods. HPLC is more sensitive than TLC and provides results with a high degree of accuracy. In order to achieve rapid results minicolumns were developed. Immunological methods were developed in late 1970's and since then many improvements have been incorporated. Immunochemical methods are more desirable than chemical methods for rapid and relatively inexpensive assay of aflatoxins.

Chapter 3: Recommended pre- season planning, pre-harvest, and harvest operations

3.1 Planting and crop management

3.1.1 Land selection

Climatic conditions: Groundnut is not suited to growing in very dry areas or at altitudes above 1500 m (around 5000 ft). Optimum temperatures are 27 - 30 °C for vegetative growth and 24 - 27 °C for reproductive growth. Between 450 mm and 1250 mm of evenly distributed rainfall is required annually for good growth and yield. Early maturing small seeded varieties require 300-500mm while medium to late maturing large seeded varieties need 1000-1200mm rainfall.

Soil selection: All soils, other than very heavy, are suitable for growing groundnut, but the best are deep, well drained sandy, sandy loam or loamy sand soils. The latter facilitate the forcing of the developing fruit into the soil (pegging). Groundnut will not grow well or fix nitrogen in acidic or infertile soils. The soils should have a pH (H_2O) between 5.3 and 7.3.

Soil testing: Utilize the results of soil tests to determine if there is a need to apply fertilizer and/or soil conditioners to assure adequate soil pH and plant nutrition to avoid plant stress, especially during seed development, which makes peanuts more susceptible to fungal infestation.

3.1.2 Soil amendments

Application of lime (0.5 t/ha), farm yard manure (10 t/ha) and cereal crop residue (5 t/ha) at the time of sowing, either singly or in combinations of lime and farmyard manure, helped reduce *A. flavus* seed infection and aflatoxin contamination in groundnuts by 50-90%. Lime, a source of calcium, enhances cell wall thickness and pod filling and decreases fungal infection (Rosolem *et al.*, 1997). Organic supplements, such as farmyard manure and crop residues, favour growth of native microbial antagonists and suppress soil- and seed-borne infections (Karthikeyan, 1996). These three components also improve the water-holding capacity of the soil, minimizing the effect of end-of-the-season moisture stress, and thereby reduce the fungal colonization and aflatoxin accumulation in the peanut seeds. Lime and farmyard manure are cheap and easily available in most developing countries, including Uganda.

3.1.3 Crop rotation

The continued cultivation of groundnuts on the same land may lead to a build-up of high populations of *A.flavus/A. parasiticus* in the soil, which will increase the probability of infection and aflatoxin contamination. A rotation of 3 years or longer can usually reduce disease, pest and weed problems. Because of the incidence of pests and soil-borne diseases, groundnut should not be grown after cotton, although cotton can be used in rotation after groundnut. Other legumes, tobacco, tomatoes and certain other vegetables may cause a build-up of nematodes and soil-borne diseases and, therefore, should be avoided in rotation

with groundnuts. Crops such as cassava, sweet potato and sunflower can also be used while crops such as maize should be avoided in rotations as they are susceptible to *Aspergillus* infection. Although a number of crops are used as intercrops with groundnut, results from intercropping research have been inconsistent, so any advantages or disadvantages are not known.

3.1.4 Seed bed preparation

Good land preparation provides suitable soil conditions for rapid and uniform germination, early weed suppression, good root penetration and growth, and steady pod formation, filling and seed development. Land should be prepared early, before the rains start, so that sowing can take place early in the rains.

All previous crop residues and weeds should be completely removed or buried, and seed beds should be smooth to provide good soil-to-seed contact after sowing. Farmers who use tractors are advised to turn the soil deep to bury residue and weeds, using a disc plough, 3 - 4 weeks before planting.

In wet, low lying areas it may be worth considering using ridges in which to plant groundnuts. The use of ridges can prevent waterlogging, and improve weed control and harvesting. Ridges should be made at, or just before, sowing and they should be flat-topped.

3.1.5 Varieties to be planted

Choose groundnut varieties which are genetically more resistant to the growth of the fungus and the production of aflatoxins, for example Serenut 2. Drought tolerant varieties also have been found to have greatly reduced aflatoxin contamination. Additionally, choosing varieties which are resistant to diseases and pests can help reduce the incidence of aflatoxin contamination.

3.1.6 Seed selection

Careful seed selection is recommended before sowing. Groundnut pods intended for seeds should be hand-shelled 1 - 2 weeks before sowing and only good quality seed should be selected for sowing. Immature, damaged, skinned, mouldy, small or shrivelled seeds should be sorted and discarded (should not be fed to animals like chicken). It is good practice to purchase certified seed at regular intervals, preferably every 2 - 3 years. The seeds must be free from contamination, irrespective of the sources of supply.

3.1.7 Seed treatment

To control seedling blights caused by soil bacteria and fungi, and also other fungal diseases, a fungicide treatment is recommended. Seeds are then treated with an insecticide/ fungicide mixture. Thiram gives good protection and can be applied as a dust at 120 g of thiram/100 kg of seed. The dust must be uniformly mixed with the seed. This will reduce seed borne infections during seedlings germination. This reduces injury to seed and allow initial vigorous growth.

3.1.8 Sowing/Time of planting

With the current weather changes in Uganda, the planting date is difficult to standardize. However, farmers should plant as soon as there is adequate moisture in the ground to ensure good germination. Select timely planting dates to take advantage of periods of higher rainfall and avoiding end of the season drought effects. Seeds should be sown at a depth of 5 - 6 cm. Seeds must not be sown immediately after heavy rains since they imbibe too much water, which causes rotting. This also results into excessive soil compaction which may hinder germination. Long duration (e.g. Igola 1, Serenut 1R) varieties should only be planted with the first rains in the first season. Short duration varieties (Serenuts 3R, 4T, 5R, 6T) can be planted in either season. Early planting generally improves yields and seed quality.

3.1.9 Plant density

Groundnut spacing depends on the growth habit of the variety. The recommended spacing ensures that there is good plant population. The recommended space between rows is 45 cm while the recommended spacing per seed is:

- bunch types: 7.5 - 10 cm (e.g. Red Beauty)
- semi-erect types: 10 - 15 cm (e.g. Igola 1, Serenut 1 and Serenut 2)

Row spacing can be reduced from 45 cm to 30 cm, if desired, and this will allow earlier ground cover and help prevent serious weed problems. Wider spacing will produce less yield per hectare. It is important to sow groundnut seed in rows and at the right spacing as this helps to reduce the incidence of rosette disease, ensures a more uniform pod maturity, better quality seed and maximizes yield. Planting groundnut plants closer together results in individual plants setting fewer pods, but over a short period of time. Overall, this will ensure that the pods will be of a similar age and stage of development and, therefore, make it easier to decide when to harvest.

3.1.10 Irrigation

Drought conditions favour aflatoxin contamination. Avoid end-of-season drought with supplementary irrigation or timely planting groundnut varieties with maturity period fitting in the rainfall cycle. Drought resistant varieties would help withstand the end or mid season drought.

3.2 Harvest management practices

Damage to pods at the time of harvest should be avoided as much as possible since this can lead to rapid invasion of the pods by *A. flavus / A. parasiticus* which leads to aflatoxin contamination. Remove excessive moisture from the pods after harvesting through shaking.

3.2.1 Timing of pulling

It is very important to harvest the crop at optimum maturity, as excessive numbers of over-mature or very immature pods at harvest can be reflected in high levels of aflatoxin in the product. Also delays in harvesting will result in poor quality seed due to mould infections and subsequent aflatoxin contamination of the seeds/pods.

3.2.2 Harvest Indicators

As pods mature, the inside portions become brown to black, while immature pods retain a fresh white appearance. The cellular layer just below the outer layer of the pod undergoes several colour changes during maturation phase. This cellular layer is called the mesocarp. It changes in colour from white to yellow to orange to brown and finally black as the pod matures. This colour distinction can be used to estimate crop maturity with the 'hull scrape' method.

- **Method 1:** ***Hull scrape method;*** Hold the pod with the beak pointing down and away from you, and with a pocket knife, scrape away the outer hull in the area from the middle of the pod to the peg attachment point. This region is known as the saddle. Pods should be moist when the colour determinations are made. To get accurate representation of the field, collect three (3) adjacent plants from 3-5 locations in the field. Collect an adequate sample for good results.
- **Method 2**: Pull 3-5 plants, strip the pods, shell and examine the insides of the shells. If the majority of the pods (70% upwards) have dark markings inside the shell and the seed are plump and the true colour of that variety, then the groundnut is mature and ready for harvest.
- **Method 3**: Use estimated period of maturity of the varieties as provided by breeder/ research institution.

3.2.3 Harvesting techniques

Two major harvesting techniques are used in Uganda; hand and hoe/ox drawn plough. Whichever method is used care should be taken not to injure the seeds and pod.

Hand harvesting/ hand pulling: Most suitable for erect/semi erect groundnut varieties (e.g. Serenut 4T and 6T) in sandy, loam soils which are well drained. Commonly used during the rainy season when the soils are moist and soft. Hold the entire crop branches as the crop is being lifted. Hand harvest only when there is enough moisture in the soil.

Hoe/Ox–drawn plough: Used for spreading groundnut varieties (Serenut 3R), on heavy soils and during dry conditions. This method is effective in lifting the entire crop from soils with a reduced pod loss. Avoid injury to pods and seed during harvesting using this method. The blades should be passed away from the pods as much as possible. A forked hoe/plough causes less pod/seed damage than unforked ones. This harvesting technique is practiced mainly in the second rains when drought usually set in at harvesting time. Mechanical damage during harvesting with a hoe is a big problem in groundnuts. When pods are damaged, the moulds will enter and produce toxins. The situation becomes worse when drying takes place on bare ground.

3.2.4 Cleaning and selection at harvests

Freshly harvested groundnuts should be cleaned and sorted to remove damaged nuts and other foreign matter. It is important to shake the plant after lifting/harvesting to remove soil from pods and avoid forming optimum conditions for the aflatoxin development. Damage to pods at the time of harvest should be avoided as much as possible since this can lead

to rapid invasion of the pods by *A. flavus/A. parasiticus*. Groundnuts should be handled as gently as possible and every effort made to minimize physical damage at all stages of harvesting and transportation procedures.
Individual plants that die from attack by pests (e.g. termites, nematodes) and diseases (wilts, pod rots, rosette) should be harvested separately as their produce is likely to contain aflatoxin.

3.3 Post-harvest Management practices

3.3.1 During and after harvest

During harvesting, transportation, processing, drying, storage and marketing, groundnut and their products may become contaminated with mycotoxins from sources such as soil, harvesting tools, processing tools, drying and storage facilities, and marketing environment. In Uganda, the traditional processing methods and practices are labour intensive and very inefficient using rudimentary tools. The risk of such contamination can be greatly increased as a result the poor traditional practices.

Recommended practices

- Timely harvesting is very important to avoid delays when groundnuts have reached maturity. The non-dormant groundnut varieties, e.g. S1, S2, S3, S4, S6 can germinate in the field especially if it rains, thus splitting the pods allowing moulds to enter.
- Immediately after harvesting, pluck the pods off the haulms and place to dry as soon as possible
- Avoid field drying of groundnuts when attached to haulms. Aflatoxins increase with delays of produce in the field (Kaaya *et al.*, 2006).
- Careful harvesting to avoid mechanical damage. This is particularly important if hand hoes are used to harvest the pods.

3.3.2 Drying of unshelled nuts

In most instances, aflatoxins are formed after harvest, particularly when harvesting takes place during end-of-season rains. The drying stage is all-important to reduce attack and damage from insects and fungi. Traditional drying techniques in Uganda involving bare ground drying (Fig. 4) are a major source of fungal contamination. They are slow, time consuming and labour intensive involving lots of crop handling, and due to rains that normally persist at harvesting, it is difficult to achieve the recommended moisture level for safe storage. Some farmers do not dry groundnuts immediately after harvest, due to labour constraints needed for plucking. Thus, they heap the nuts either in the field or in houses (Fig. 5). Sometimes farmers store wet groundnuts in bags for a few days waiting for sunshine (Fig. 6) These practices, coupled with inefficient and slow drying process under the humid conditions enhance aflatoxin contamination greatly.

Recommended practices

- Do not dry produce in contact with soil. Use clean sheets, for example polythene sheets, or tarpaulin or mats made of papyrus, cemented grounds or raised structures (Fig. 6b)
- Dry groundnuts as soon as possible (in developed countries, drying is within 48 hrs)
- Sun dry grain to bring down its moisture below 13%
- Do not dry diseased/infected produce along with health ones.

3.3.3 Shelling

Mechanical damage to foodstuff during shelling, threshing and winnowing makes them much more vulnerable to invasion by storage moulds, including *A. flavus*. Under any given environmental conditions fungal growth may be several times faster in damaged compared to intact nuts. Cracks and breaks in groundnut pods and testa are caused mainly during shelling by trampling or use of machines. There are two types of groundnut shellers now used in Uganda. The hand operated (Fig. 8a) and the motorised shellers. The latter normally use electricity and can be a simple type that can handle small volumes of groundnuts (Fig. 8b) or big type that handle several bags of groundnut per hour (Fig. 8c).

Recommended practices

- Separate out immature pods as well as those infested with pests and diseases
- Do not shell by beating or trampling on groundnut in shells
- Manual or motorised shelling is recommended but the shellers should not damage the pods.
- Use hand or motorised shellers specifically designed for groundnuts
- Do not sprinkle water on dry pods while using mechanical shellers. Instead, adjust (where possible) the space between blades and the sieve according to pod size to reduce breakage.
- Remove shrivelled, discoloured, mouldy and damaged grains from the lot including groundnuts with damaged testa and put them in new polybags.
- Remove dust, and foreign material which can provide source of contamination

3.3.4 Postharvest Storage

The fundamental reason why groundnuts should be stored dry is to increase storability and in part, prevent growth of storage fungi. If groundnuts are stored incorrectly, that is, in an improperly dried state or under high humidities with inadequate protection, fungi will inevitably grow. Duration of storage is an important factor when considering aflatoxin formation. The longer the retention in storage the greater will be the possibility of building up environmental conditions conducive to *A. flavus* proliferation and production of aflatoxin.

In Uganda, groundnuts are stored in two forms: In shells/pods (unshelled) and in shelled form (as kernels). The former method of storage is mainly at farm level (Fig 11), while the latter form of storage is at retail level (Fig 12) since groundnuts are delivered to wholesalers and retails already in shelled form. Storing groundnuts in shells/pods is more recommended because shells offer protection against mould infection. When stored in kernel form, groundnuts deteriorate very fast because they pick-up moisture and are easily invaded by moulds, insects and rodents.

In most parts of Uganda, however, traditional means of crop storage are not yet improved as evidenced by the storage structures (Fig 11), whether traditional or modern, should maintain an even, cool and dry internal atmosphere; they should provide protection from insects, rodents, and birds; should be easy to clean and should be water proof and protected from flooding. These recommendations were made in view of *A. flavus* infection and aflatoxin production in stored groundnuts and other produce.

The maximum moisture content for storage of groundnuts (unshelled) is 9% while that for shelled groundnuts is 7% (Odogola, 1994; Waliyar *et al.*, 2007; 2008). At these moisture contents, if the relative humidity is maintained at 70% and temperature 25 – 27^{0}C, there is guarantee for safe storage of the nuts for approximately one year.

Recommended practices

- Properly dry groundnuts to safe storage moisture content
- Groundnuts should be placed in packages that will maintain suitable environment and prevent or restrict moisture pick-up and insect/rodent infestation.
- Use new/clean gunny or polybags to store the groundnuts.
- Put only clean sorted kernels into the bags
- Bags should not be placed directly on floor
- Do not heap groundnuts in shells/pods on the floor/ground inside storage structure
- Maintain proper storage facilities (well-ventilated, dry and low relative humidity) and care not to expose produce to moisture during transport and marketing.
- Control insect and rodents during storage
- Do not mix new with old stock produce

3.4 Processing groundnuts into products

It is important to note that groundnuts used for product development must be clean and free from mould and aflatoxins. Moulds can have adverse effects on the flavour and appearance of groundnut products. The most common off-flavour of mouldy groundnuts is the bitter taste. Groundnuts can be processed into a number of products. These include roasted nuts, peanut butter, peanut flour, peanut paste, peanut oil, peanut milk, peanut biscuits, bio diesel and animal feeds among others. Groundnuts can also be used as an ingredient in making products like chocolate, energy bars, cakes; and can be coated with flour to make several deep fried products. It can be mixed with simsim (sesame) to make a composite paste commonly used to make stew in Northern and Eastern Uganda.

Flour/paste: In Uganda, it is now a common practice to process groundnuts into flour or paste and these are sold in retail shops. The flour or paste is commonly processed from

groundnut rejects which include broken, shrivelled and mouldy nuts delivered to the market mixed with sound kernels. Use of broken, shrivelled and mouldy kernels is very dangerous because these have the highest contamination levels of moulds and aflatoxins. Up to five different types of groundnuts products can be processed from these rejects (Kaaya *et al.*, 2006). They are the flour, the pressed type, the light brown paste, brown paste and dark brown paste (Fig. 13).

The flour is processed from un-roasted groundnut rejects using a hammer mill and are sold packaged in the usual polypropylene bags used for holding other products like maize, rice, beans and coffee. This is inadequate storage which leads to rancidity, mould proliferation and aflatoxin production. The pressed, light, brown and dark brown pastes are processed using a blender type of machine locally fabricated and these products differ in colour and texture depending on the degree of milling since the blender roasts at the same time. The dark brown paste is normally milled for a much longer time. Since the machines are locally fabricated and not made out of stainless steel, they wear and tear leaving dangerous particles in the products. In fact, processors replace the blades within a short time depending on how often the machines are used. Thus, a combination of aflatoxin content and the worn-out particles make these groundnut products quite unsafe for human consumption.

The majority of the products processed from rejects are heavily contaminated, with the flour type having the highest levels while the dark brown paste has the least levels (Table 4).

Table 4: Mean aflatoxin levels (ppb) in different groundnut forms from markets

Groundnut form	District			
	Iganga	Mayuge	Mubende	Kampala
Unsorted kernels	80	70	61	65
Sorted kernels	38	25	27	29
White flour	70	66	56	64
Pressed	54	53	35	39
Light brown	45	33	30	32
Brown paste	38	32	27	27
Dark brown paste	34	25	23	28

Adapted from Kaaya et al. (2006)

Peanut butter: In Uganda, there is both local and imported peanut butter (Fig 14). Peanut butter can be applied on bread like margarine, can be added to green vegetables, beans, dry fish then eaten as sauce with food, can be eaten with ripe bananas and if desired can be added to any type of food.

The process of making peanut butter starts with roasting dry raw groundnut kernels. The raw groundnuts are first sorted to remove the split, mouldy and shrivelled nuts and any stones or foreign matter. Sorting and cleaning helps to reduce chances of aflatoxin contamination

in the final product. During roasting, nuts are exposed to dry heat at a temperature of 120 - 150°C or onwards, depending on nuts. The process adds value by imparting flavour and aroma to the nuts. The process is also very important in the initiation of processes for oil/fat extraction. After thorough roasting, the following steps are followed.

Recommended step by step processing method for peanut butter

- Remove the top of the groundnuts cover (called skin) if you desire.
- Place in the grinder or blender, the quantity depends upon the size of the blender and how much butter one needs to process.
- Grind until the groundnuts turn to a paste.
- Open the grinder cover and use a wooden spoon to mix.
- Continue grinding until a smooth consistency is formed and you don't see any more broken kernels
- Pour into the containers and cover properly.

3.5 Packaging and storage of groundnut products

3.5.1 Packaging

The packages or containers used to store groundnut products should be able to exclude air and moisture pick-up. If air enters, it will accelerate the process of rancidity which induces off-odours and off-flavours while moisture will encourage mould growth and subsequent aflatoxin production.

The containers may be plastic or glass which should be covered thoroughly to avoid entry of air and moisture. For peanut butter, plastic or glass jars are recommended. These should be sealed tightly to avoid leakage of oil. Oil separation is common in this product and, quite often, if not well-sealed, containers leak and the oil affects the appearance product including the label. The locally processed peanut butter or paste in retail markets of Uganda is commonly packaged in polyethylene bags (Fig 14) which are manually tied. This does not offer maximum protection against moisture entry and leakage. Some traders use plastic containers of different shapes with tight covers which is a good practice. It is important that the containers are well-labelled to include all the important information desired by the consumer and Uganda National Bureau of Standards (UNBS).

3.5.2 Storage

Storage of all processed groundnut products should be in a cool dry place. In retail markets, locally processed peanut butter and other products are not stored properly, often exposed to sunshine. The heat produced accelerates rancidity and also encourages growth and multiplication of micro-organisms including moulds and bacteria like Salmonella. Salmonella contamination of peanut butter has been a major problem in the United States of America (USA) and several tonnes of peanut butter have been recalled from supermarkets. It is therefore important that we improve the quality of the nuts used to produce the products and also maintain recommended packaging and storage procedures.

3.6 Shipping of groundnut products

It is important that during shipping, containers of groundnut products are well-protected. They should not be damaged to allow air and moisture absorption and the environment should be cool. It is therefore important that rigid secondary packages/containers are used to hold primary containers where the products are packaged. For example, the jars can be packed in paper cartons (boxes) which are then stacked in the vehicle for transportation. Under such circumstances, in-pack movements of the jars should be avoided.

Transportation of shelled groundnuts in Uganda is a serious problem. The nuts are packaged in polypropylene bags which quite often are not well-sealed. Thus, spillage is a common phenomenon. The trucks are not well-covered and thus sometimes the bags are soaked with rain. It is important that the trucks are well-covered with tarpaulins/canvas to avoid moisture and dust.

Recommended practices for traders

- Always purchase new or current season produce. Avoid purchasing produce stored for more than six months.
- The company should establish its own purchasing system rather than dealing with middlemen. This may involve purchasing groundnuts directly from farmers.
- Always check groundnut quality condition before acceptance. These include:
  - Moisture content (not more than 13%); use moisture meters
  - Presence of diseased/mouldy and discoloured grains
  - Presence of broken kernels
  - Presence of soil contaminated grain
  - Presence of foreign matter (soil, dust, chuff and stones).
  - Presence of insects and insect damaged grain.
- Sorting and cleaning to remove the above-mentioned poor condition kernels should be done prior to processing. Do not process poor quality kernels because garbage in, garbage out. ***This behaviour is common for groundnut processors and hence their products are heavily contaminated with aflatoxins***
- Unshelled groundnuts (those still in shells/dry pods) are better to purchase than shelled nuts since the shell protects them against mould invasion.
- If kernel moisture content of delivered nuts is greater than 13%, (but should not be more than 15%) immediate further drying of produce should be done on a clean surface, to reduce moisture content to 9%.
- Groundnuts purchased should not be stored for more than three months.
- The nuts should be stored in new interwoven polypropylene bags stacked in a moisture and rodent proof store (no rodents and insects)
- The store must be routinely cleaned to remove spilled kernels and other foreign materials.
- Proper packaging of the products in moisture-tight containers is recommended.
- On-spot mould and aflatoxin tests in the raw and finished products must be carried out.
- Processors should strive to get the Quality Mark from the Uganda National Bureau of Standards.

Chapter 4: Novel/future approaches and awareness building

4.1 Host plant resistant breeding

Past research has identified and developed groundnut varieties that are resistant to *Aspergillus flavus* invasion and subsequent aflatoxin contamination. In Uganda, some of these lines are undergoing on-station trials to identify superior ones. Groundnut farmers will be exposed to these varieties through participatory on-farm trials/demonstrations. Long term plans are underway to transfer these resistances into popular/desired groundnuts varieties.

4.2 Biocontrol options for *Aspergillus* Spp

Biocontrol of aflatoxin contamination is a promising technology. A biopesticide, consisting of a rhizosphere-competent non-aflatoxigenic strain of *Aspergillus* with competitive saprophytic ability, may competitively exclude toxic strains from infecting the crop.

Fluorescent pseudomonads and several strains of *Trichoderma* species inhabit the rhizosphere of many crop plants and have been identified as potentially promising biocontrol agents against *A. flavus.* Since the beginning of the 21st century, a large number of *Trichoderma* (>250) and *Pseudomonas* (> 100) isolates have been obtained from groundnuts rhizosphere and evaluated for their antagonism towards *A. flavus* and their ability to reduce preharvest kernel infection of groundnuts. Significant reduction of *A. flavus* populations and kernel infection occurred in both greenhouse and field experiments. Two *Trichoderma* isolates, Tv 47 and Tv 23, and two bacterial isolates *P. cepacia* (B 33) and *P. fluorescens* (Pf 2), were effective in reducing aflatoxin content in the kernels. The effectiveness of the biocontrol agents still needs to be established under African field conditions and simple, cheap and effective formulations developed for use in farmers' fields. Integration of these biocontrol agents with host plant resistance and agronomic management would provide an environmentally-friendly option for the management of aflatoxin contamination in groundnuts.

4.3 Biotechnological approaches

Biotechnological approaches to increase host plant resistance through the use of anti-fungal and anti-mycotoxin genes also have begun. This approach received a major boost with the successful establishment of groundnut regeneration and transformation protocols, and led to the transformation of groundnut with a rice chitinase gene to help prevent invasion by fungal pathogens. These transgenic events are now in advanced generation, with some events showing good resistance to *A. flavus* infection (< 10% infection) in *in vitro* seed inoculation tests. Such events can be used in conventional breeding to develop agronomically superior peanut varieties that are highly resistant to aflatoxin contamination.

4.4 Enterosorption and chemoprotection

Researchers have developed mechanisms of detoxifying aflatoxins once consumed by animals. Chemoprotection against aflatoxins involves use of compounds that either increase the animal's detoxification process or prevent the production of compounds that cause damage to various parts of the body. The first progress was the discovery that certain zeolytic minerals could selectively adsorb aflatoxins tightly enough to prevent them from being absorbed through the intestine (Harvey *et al.*, 1993). The utilisation of specially processed phyllosilicate/bentonite clays especially hydrated sodium calcium aluminosilicate (HSCAS) that selectively bind and inactivate aflatoxins in the gastrointestinal tract of farm animals such as chickens, turkey poults, lambs, goats and pigs has been the most successful chemoprotection (Phillips *et al.,* 1995). These clays have been recommended to be incorporated in animal feeds as additives that provide protection from the toxins (Devegowda *et al.*, 1998).

Following discovery of petroleum in Uganda, the search for bentonite prospects was mounted all over the country by the Department of Geological Survey and Mines (DGSM) because this mineral is used during the drilling process. Subsequently bentonite prospects were discovered in Kaiso-Tonya, Nkondo-Ssebugoro, Butiaba, Buseruka, Kibiro, Katwe, Kibuku, Muhokya and Rwimi. Bentonite prospects were also discovered by Mr. Prosper Ndyabahika in Burama and Ntungwa, in Rukungiri and Kanungu Districts. There are therefore chances of conducting studies on the possibility of using this bentonite to fight against aflatoxin poisoning of humans and animals in the country.

4.5 Forecasting

Establishing a prediction system to forecast the likely risk of aflatoxin contamination before or during the cropping season would facilitate implementation of appropriate management practices by the farmers. Development of these forecasting models requires quantitative data on the relationships amongst environmental and crop management factors, *A. flavus* infection and preharvest aflatoxin contamination. For broad-scale applications these models should be based on remote soil, water and temperature monitoring. The availability of such prediction models will help target improved aflatoxin management technologies on a regional scale.

4.6 Promoting public awareness and enforcement of standards

The other strategy to manage aflatoxins is to promote awareness and enforcement of standards. This can be achieved through:

- promoting mycotoxins (aflatoxin) hazard campaigns through training especially the smallholder farmers
- Developing and execution of programmes to disseminate the information about aflatoxin hazard particularly to the most remote villages through radios, pamphlets, posters and fact sheets
- Public information campaigns about the importance of consuming only good quality groundnut products and effective crop harvesting, processing, drying and storage (shelf life) should be conducted

- Monitoring exercises for incidents of mould growth should be carried out. Banning consumption and sale of moulded groundnut products should be done
- Capturing the attention of millers, traders, policy makers, schools, religious institutions, and community development bodies can be a good strategy. Seminars and field days are good avenues to exchange experiences and to get acquainted with progress by other players
- Use good /sound hygiene rule like clean water, drying, processing, storage containers, among others must be followed regardless of the crop being processed
- It is also recommended that regulatory standards on mycotoxins in the country be appropriately developed, maintained and enforced.

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