STUDIES ON THE CONTROL OF FUNGAL CONTAMINATION AND AFLATOXIN PRODUCTION BY *ASPERGILLUS FLAVUS* LINK IN A CEREAL GRAIN BY THE COMBINATION TREATMENT OF HEAT AND IRRADIATION

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STELLINGEN (THEOREMS)

1. Moulds contaminating stored cereal grains and grain products belong predominantly to the genera *Aspergillus* and *Penicillium*. *Aspergillus* spp. and *Penicillium* spp. impart potent mycotoxins into stored produce.
The contaminating mycoflora exhibits four patterns of infection and although some cannot be isolated after 4-5 months storage (e.g. *Penicillium digitatum*, *P. expansum*, *Fusarium moniliforme*, *Paecilomyces varioti*); they would have already released the toxin into the food.

2. The conventional aerial prophylactic spraying of stored grains and cereals with fumigants and insecticides temporarily reduces aerial spores of fungi. On each occasion of spraying, the aerosol is inhaled by the workers; the chemical burden on grains becomes additive as more spraying is carried out at a later date.

3. Insecticides do not kill fungi. The contaminating moulds reappear after a lapse of a few days in large numbers.

4. High tropical environmental conditions for most part of the year (>85% R.H. and 28±3 ^{o}C) are conducive for rapid proliferation of fungi on stored cereal grains and grain products as the moisture contents (m.c.) increases above the stipulated safe m.c. for storage. Insect activity (producing metabolic water) combines with high ambient R.H. to induce mould growth. In addition, insects ingest and carry mould spores on their bodies and droppings and spores may spread to distances as far as an insect can go within a storage sack.

5. A combination of moist heat (60 ^{o}C for 30 min.) and irradiation (4.0 KGy) can decontaminate maize of fungi. This result could be extended to other cereal grains and grain products.

6. Unlike dry heat, moist heat has high penetrating power, increasing target size and causing rapid coagulation of protein.
The general mutational effect of irradiation is to cause damage to the genetic and biological mechanisms, creating impairment of function.

Dedicated to my parents, and to my beloved wife Catherine, who through the years, through thick and thin, has lovingly and patiently encouraged me to pursue God's best for my life and profession.

ABSTRACT

Traditional storage of maize in tropical countries such as Ghana results in the rapid development of numerous fungi, including potential mycotoxin producers such as *Aspergillus flavus* (aflatoxins), *A. ochraceus* (ochratoxins, penicillic acid), *Fusarium moniliforme* (moniliformin), *Paecilomyces varioti* and *Penicillium expansum* (patulin). Treatment of maize with a combination of moist heat (30 min. at 60 °C and relative humidity >85%) and gamma irradiation (4.0 kGy) proved to be effective in inactivating the resident population of fungal spores. This result was confirmed by *in vitro* studies with spores of *Aspergillus flavus* NRRL 5906. In a comparative study of packaging materials it was found that food commodities stored in woven polypropylene bags for six months at 85% R.H. had mould and yeast counts which were 2-3 log cycles lower than those of products kept in jute bags. Also, the viability of the seeds was better preserved in polypropylene sacks.

It is recommended that the combination treatment be carried out on good quality grains, and that woven polypropylene sacks are used in bagging prior to irradiation, for maximum extension of shelf-life.

CONTENTS

INTRODUCTION

Maize (*Zea mays* L.) has been under cultivation for several thousands of years in the Americas, but it was Columbus who introduced it in the early 1500's into West Africa where its uses have increased.

Maize, an example of cereal grain is a very important staple food crop in Africa and is as a rule stored for long periods, as buffer stock, for both human consumption and lately as ingredient for poultry and livestock feed. Maize production in Ghana is primarily for human consumption and a large proportion of the population (>70%) depend on maize as the principal source of food. Indeed, as many as twenty different kinds of food can be prepared from maize (Eyeson and Ankrah, 1975).

The crop is cultivated throughout the country with varying degrees of success, depending on the edaphic and climatic factors. In Ghana, the areas of maize cultivation include the whole of Southern Ghana, Ashanti, Brong Ahafo, the Northern Region and parts of the Upper Region. The most suitable soils which enhance maize production and their distribution are the Kumasi, Asuansi, Kroso, Swedru, Nsaba, Bekwai and Kokofu series found in the forest areas of Ashanti, Central, Eastern, Western and Brong Ahafo Regions. In the transitional zone, Bedeisi and Sutuwa series in the Brong Ahafo, Central, Ashanti and Eastern Regions are also very suitable for maize cultivation. The most suitable soils in the Savannah Vegetation Zone are the Damongo, Ejura, and Valempele series found in the Upper and Northern Regions and parts of Ashanti. Suitable soils of Coastal Savannah thicket are the Oyarifa, Toje, Abouku and Bajiase series among several others. In the Volta, Eastern and Central Regions, the Afeyi, Kpandu and Asokwa series form the suitable soils for maize cultivation.

Intensive commercial production of maize in Ghana is however found in four main centres: (a) the Somanya District of Eastern Region; (b) the Midland Maize belt of Ashanti and Brong Ahafo Region; (c) the Ho-Kpandu district of Volta Region, and (d) the Swedru-Adjumake District of the Central Region.

Elsewhere substantial amounts may be produced but these are used mainly for home consumption on a subsistence economy and do not feature in commercial estimates.

Being a seasonal crop, especially in West Africa, maize is stored as dry grains and forms an enormous reserve of food, However, substantial amount in storage are subject to attack by a variety of insects, fungi and other biodeterioagents. Losses in storage due to insects and fungi is estimated at between 30-50% of annual harvest (Rawnsley, 1970; Adams, 1977). In the recent 1983 estimates by the Ministry of Agriculture in Ghana, they put losses owing to insect and fungi activity at 60 million in local currency. Production and Import figures for maize (1970-80) is presented in Table 1.

The role of fungi in quality deterioration of grains is well documented in developed countries (Bothast et al., 1975; Christensen and Kaufmann, 1965, 1969; Lagrandeur and Poisson, 1968; Semenuik, 1954; Tuite, 1978; Tuite et al., 1967, etc.) but there is paucity of information regarding these fungi on maize grains in West Africa and the role such fungi play.

Broadbent et al. (1969) provided an extensive list of fungi associated with maize isolated in Nigeria. Later, Oyeniran (1973a,b) added eleven different fungi belonging to the genera *Aspergillus* and *Penicillium* to the list. Other workers in Egypt (Moubasher et al., 1972) isolated and identified thirty-four species of fungi belonging to thirteen genera on stored maize. Members of the genus *Aspergillus* were the most predominant followed by *Penicillium*. Danquah (1973) isolated twenty-two seedborne fungi on maize in Ghana including only one *Aspergillus* sp. and one *Penicillium* sp. and he showed the pathogenicity of some of them to maize seedlings. He did not, per se, report of any toxigenic species of potential human hazard.

Fungi infect grains in the field before harvesting or during harvesting through pure mechanical damage or in storage by aerial spores in the storage silos or bins.

Losses induced by fungi and insect activity

The major type of losses caused by fungi growing in stored grains are (i) decrease in viability (ii) discolouration of part (usually the germ or embryo) or all kernel or seed (iii) heating or mustiness (iv) various biochemical changes (v) loss in weight (vi) production of toxins that if consumed may be injurious to man and domesticated animals. These are exhaustively discussed by Christensen en Kaufmann (1969).

The role of storage fungi as a source of insect food and their effect on the breeding of some stored products insects e.g. *Sitophilus oryzae* has been reported (Agrawal et al., 1957; Misra et al., 1961; Sikirowski, 1964; Widstrom, 1979). Results indicate that larvae, pupae and adults of various stored products insects carry toxigenic and non-toxigenic species of fungi, internally (Table 2). The major economic loss caused by grain-infesting insect is not always the actual material they consume, but also the amount contaminated by them and their excreta which make food unfit for human consumption. Suffice to say that the cumulative effect of insect feeding, breeding, transmission of toxigenic and saprophytic fungi and associated changes in the microecological conditions in the bulk grain, hasten the microbial deteriorative process.

Table 1. Production and imported supplement of maize in Ghana for the period 1970-1980.

Year	Production figure (thousand metric tonnes)	Import supplement (thousand metric tonnes)
1970	483.0 [a]	529.0 [b]
1971	465.4	338.0
1972	402.4	2.0
1973	427.0	242.2
1974	486.0	26.0
1975	343.0	44.0
1976	295.0	105.7
1977	299.0	
1978	386.0	
1979	370.0	
1980	424.0	

Source: a) Ministry of Agriculture, Ghana, Planning and Research Division, Accra;
b) Central Bureau of Statistic, Accra.

The activity of infesting insects make metabolic water available which goes to cause marked increases in the moisture content (m.c.) of grains. For each of the common species of fungi infecting grains, there is a minimum m.c. in grains below which the fungus cannot grow. These minimum m.c. have been determined for most common storage fungi growing on starchy grains (Christensen and Kaufmann, 1969). Davey and Elcoate (1965) put the safe m.c. for storage of maize at about 13.0% whilst Lutey and Christensen (1963) previously suggested that viable field and storage fungi may be generally reduced or eliminated from grain lot at m.c. 12-14% and a temperature of 20-30 ^{o}C, without serious deterioration of grains. It is the interseed Equilibrium Relative Humidity (E.R.H.) expressed as a fraction that determines fungal activity (Christensen, 1974, 1978; Koehler, 1938; Semenuik, 1954). This fraction or water activity a_w is the

Table 2. Fungal species isolated from storage insects.

Insect species screened	Toxigenic species	Other species
Sitophilus aryzae, Linn.	*A. flavus, A. candidus, A. ochraceus, A. fumigatus*	*A. sydowi, A. ruber, A. niger, A. chevalieri, P. rugulosum, Amblyosporium* sp., *Cladosporium* sp.
Tribolium castaneum Hebrst.	*A. flavus, A. candidus, P. islandicum*	*A. versicolor, A. niger, A. ruber, A. chevalieri,*
Trogoderma granarium Everts	*A. flavus, A. candidus, P. islandicum*	*A. ruber, A. niger, A. glaucus* gr., *A. sydowi, A. versicolor, P. restrictum*
Callosobruchus chinensis Linn.	*A. flavus, A. candidus*	*A. ruber, A. sydowi, A. glaucus* gr.
Oryzaephilus surinamensis Linn.	*A. flavus, A. ochraceus*	*A. restrictus, A. terreus, A. glaucus* gr., *P. decumbens, Cladosporium* sp.
Stegobium paneceum Linn.	*A. candidus*	*A. glaucus* gr.
Rhizopertha dominica Fab.	*A. candidus, A. ochraceus*	*A. niger, A. glaucus* gr.
Araecerus fasciculatus de Geer	*A. flavus, A. candidus*	*A. niger, A. glaucus* gr.
Corcyra cephalonica Staint.	*A. flavus, A. candidus*	*A. sydowi, A. ruber, A. niger, A. restrictus, A. versicolor, P. spinulosum, P. corylophilum, Nigrospora* sp.
Ephestia cautella Walker	*A. flavus, A. candidus*	*A. niger, A. glaucus* gr. *A. terreus, A. ruber, A. versicolor.*

ratio of the water vapour pressure of substrate to the water vapour of pure water at same temperature and pressure (Scott, 1957). Water activity has now taken the place of moisture as the most useful expression of the availability of water for growth of microorganisms (Scott, 1957). The a_w of food product is low (i.e. low m.c.) when the solute molecules bind water molecules. Then, the water molecules are less free to escape from the surface of the food product into the vapour phase, and the vapour pressure is low. When bound, water molecules are not available for fungal growth. Fungal growth is therefore related to the a_w. Indeed, it is believed that storage fungi do not readily grow below an E.R.H. of 75% (a_w 0.75) (Christensen, 1978).
Unfortunately, the environmental conditions in the tropics with high average humidity $\geq$80% R.H. and high temperatures (28±3 °C) favour all kinds of deteriorative processes of stored grains.

Odamtten and Langerak (1980) showed that the equilibration period of two varieties of maize (Ghanaian local variety and Dutch yellow maize) held under R.H. 60-80% was 12 days after which there was no further increase in m.c. The m.c. of grains kept at 85-95% R.H. continued rising above the stipulated safe m.c. for storage of grains (11-13%). Gain in weight owing to water sorption is at the expense of the quality of grains, whose shelflife is shortened by the growth of fungi on such grains. The equilibrium moisture content (EMC) of grains is influenced by genotype (Hubbard et al., 1957), previous drying temperature (Tuite and Foster, 1963), storage temperature (Pixton and Warburton, 1971), hysteresis (Hubbard et al., 1957) or whether grain is ground or unground (Tuite and Foster, 1963). However, Odamtten and Langerak (1980) found that moisture sorption isotherms (at varying humidities) obtained for Ghanaian local maize variety were akin to that obtained with Dutch variety maize except that the Dutch variety was less hygroscopic, absorbing comparatively lower water vapour moisture at high storage humidities and containing 14.0% m.c. at 80% R.H. (a_w 0.8) as compared to 15-17% m.c. for the Ghanaian variety under the same humidity (80% R.H.) condition. They concluded that control and storage conditions which would be ideal for storage of Ghanaian local maize would also be suitable for the Dutch variety and vice versa.

Mycotoxin contamination of foodstuffs

Foodstuff are contaminated with mycotoxins in different ways. Toxigenic moulds may grow and produce mycotoxins in agricultural produce in the field as well as during transport and storage when conditions are favourable i.e. at high

$a_w \geq 0.70$. Berman (1958) and Higginson (1963) showed that the areas showing high incidence of primary cancer of the liver include the sub-Sahara region of Africa (where Ghana is located) and Southeast Asia. Interestingly, these are all areas of continuous high humidity and high to moderate temperatures in general. These climatic conditions favour mould growth on such stored staple foods as rice, maize, rye and groundnuts. The mycotoxin problem in these areas is becoming serious; *Aspergillus flavus* and most other toxigenic moulds are extremely common and have been found to grow on a variety of foodstuffs under a wide range of conditions.

Extensive field investigations concerning contamination of human foodstuffs with mycotoxins have been conducted in many African, and Asian countries including South Africa and Swaziland (1966-1969) as reported by Gilman (1971); Uganda (1965-1967) reported by Crawford (1971) and Alpert et al. (1971); Thailand (1967-1970) reported by Shank (1971); Mozambique (1969) reported by Purchase and Gonsalves (1971); Philippines (1967-1969) reported by Campbell and Salamate (1971); Kenya (Peers and Lindsell, 1973); Ghana (Lokko, 1978). New Guinea, Korea and Japan. Results of the field investigations on isolation of toxic fungi from foodstuffs showed that most of the fungal species belong to the two major genera of storage fungi, *Aspergillus* and *Penicillium*. These foodstuffs included rice grains (unhulled, rough and polished rice) other cereal grains (wheat, barley) maize, soybean, red beans and other legumes, cassava flour, fermented foods such as miso (soybean paste), shoya (soya sauce) dried fish and pickled vegetables (mukamiso). Aspergilli and Penicilli are mostly confined to stored products because of their adaptation to dry conditions. Fusaria thrive in wetter conditions.

Interestingly, no significant difference was observed in the kinds and distribution of fungi among the investigated areas of the world (Purchase, 1971), though there are several characteristic patterns of mycoflora distribution in each local area and in each type of foodstuff. Some work has been done in Ghana with regard to the fungi that infect foodstuffs. Lokko (1978) provides the first information on aflatoxin in cassava flour. There are also sporadic reports of death of poultry and human beings as a result of ingesting maize, cassava flour, animal feed etc. contamined by fungi and Food Research Institute has record of aflatoxin presence in maize (unpublished data). This is because the recommended practices for prevention of mycotoxin formation in food, feed and their products by the FAO is not strictly adhered to by some farmers and secondly our ambient environment naturally promotes fungal deterioration. Drying of the produce is an effective means to prevent further growth. However, if mycotoxins have been formed, the process of contamination can be stopped but not reversed by drying. Because of their stability, mycotoxins may pass industrial processing of the produce without large reduction and enter into consumer products (Harwig et al., 1974; Marth and Doyle, 1979; Van Egmond et al., 1977). Aflatoxins themselves, or derivatives of them have been detected in the urine, faeces, and milk of animals, and even in human urine (Campbell and Salamat, 1971). Smith et al. (1976) showed that incidence severity of aflatoxicosis is great when there is poor on-farm storage of maize and that aflotoxin formation continues during and after milling process.

Aflatoxins formed by *A. flavus* and *A. parasiticus* are the most studied mycotoxins because of their well known carcinogenic and etiological effect of human and animal malignancies (Rodrick and Stoloff, 1977). Aflatoxins showed carcinogenic target effect on organs other than the liver, such as the stomach, kidney, trachea and intestines (Butler and Barnes, 1966; Dickens et al., 1966).

Zuber and Lillehoj (1979) recently reviewed the status of the aflatoxin problem in maize and stated that conditions of stress such as drought have been shown to enhance aflotoxin contamination problem. If this is true, then the West African region stand a great risk of quality deterioration of their little stock of maize at this material moment of acute drought conditions. Prior to 1971, aflatoxin contamination in maize was associated with stored grain (Christensen and Kaufmann, 1968). Afterwards, it was demonstrated in pre-harvest maize (Anderson et al., 1975). Since most of the maize grains or their flour are frequently used in food or feed, it became necessary to develop methods to prevent toxin formation.

Application of conventional technology for preservation

Sun drying is a cheap form of natural energy used in reducing the m.c. of maize-on-the-cob and hulled maize to safe (12-14% m.c.) i.e. an a_w of 0.70-0.75 for storage. In Ghana, the traditional platforms, and mats used for sun drying of shelled maize are variable from one locality to the other. In some instances (also in the Nigeria, Kenya and Zambia) grains are left to mature and dry on the cob in the field. The maize grains are collected from the field when they are completely dry for threshing. Another common practice is to cut the stalks at the base and arrange them in bundles or layer in the field and on barns at home, a process referred to as stooking. They are then allowed to dry in the sun. The maize cobs may also be tied together by using the husks and can then be hung on bundles from staves in a traditional fire-place of a kitchen (to promote smoke infiltration into the bundles supposedly to kill insect and fungi) or from a tree as in the case of Zambia (Chalwe, 1980). Admittedly, mould growth is still possible because of the increased grain temperature and the attendant insect activity making available metabolic water for fungal growth. Moulds such as *Aspergillus flavus* can invariably be detected (Converse et al., 1978b).

High temperature drying is the method of choice in conditioning maize for storage and marketing. Grain dried at high temperature is decreased in hygroscopicity and as a result is in equilibrium with a higher R.H. than low temperature dry grains (Tuite, 1979): Dryers, particularly the cross flow and batch driers, operating at too high a temperature and high drying rate give brittle kernels caused by the formation of stress crack in the endosperm (Peplinski et al., 1975; Thompson and Foster, 1963). As a result, broken maize foreign matter is increased and this constitutes a storage hazard because they are hygroscopic and the surface is exposed: acting as a food bait for fungal spores. Such broken grains contain 6-7 times as many spores as their unbroken kernels (Tuite, 1975).

Storage situation in Ghana

Hermetic storage of high moisture maize practiced in developed countries was the innovative venture of Vayssière (1948) in France. The grain undergoes fermentation, oxygen is depleted and carbon dioxide is increased by the respiration of grain yeast and bacteria (Burmeister at al., 1966; Isaacs et al., 1959). Areas with warmer climates and much rainfall in the tropics, like Ghana are more subject to insect and mould contamination of stored grains. Variable traditional storage bins used in different sectors of Ghana have been collated and reviewed by Nyanteng (1972). In addition to these traditional structures, concrete, plywood, aluminium and butyl silos are mostly used and some bulk grains are stored in jute sacks in open cement warehouses. The location of concrete and butyl silos storage facilities in Ghana for cereal grains and animal feed have been reviewed in a recent report (Anon, 1979). The storage facilities in the country was considered inadequate because functional concrete silos could store a 69,000 metric tonnes capacity of cereal grains whilst butyl silo could contain 130,544 metric tonnes of cereal grains. Meanwhile, the total amount of imported cereals and pulses needed to be stored by 1976 was 635,819 metric tonnes. Such a situation has favoured the handling of over 60% of the grain surpluses or supplies by farmers (majority being tradition farmers) outside those specialized storage facilities. Insect and fungal deterioration of grains under such conditions become the rule rather than an exception. Studies have indicated that hermetic or air-tight structures are better than non-air tight structures for grain storage (Hyde and Okley, 1960). But in silos and storage bins under tropical conditions, even with 10% initial moisture content, thermogenesis, discolouration of grains and fungal growth develop within weeks of storage. Turning of the maize grains to promote greater uniformity in moisture distribution, blowing dry air through the grain mass, and other mechanical handling are required that are difficult and expensive. Imported bulk grains are bagged in jute bags and then sent to open cement warehouses with appropriate roofing. But the grains are sooner or later exposed to the high ambient temperatures ($\geq 28\ ^{\circ}C$) and humidity ($\geq 80\%$ R.H.) and they in no time attain a_w which promote both insect and fungal growth. To prevent deterioration in storage warehouse agents and private farmers carry out regular prophylactic spraying with recommended insecticides and fumigants.

Chemical control

The use of chemical sterilants for the control of post-harvest diseases of insects and fungi is well known. For large scale fumigation, methyl bromide or mixtures of ethylene dibromide and methyl bromide (1:3 at 32 g/m^3) has proved to be effective against insects and fungi when applied under tropical conditions. In non-airtight warehouses, fumigation sheets made of balloon film, Poly Vinyl Chloride (PVC) coated aluminized fabric or high density polyethylene laminated with nylon, are sufficient to retain these fumigants during exposure periods. Phosphine generating fumigant products of Detia(R) X are also widely used in Ghana. In order to control any infestation present either in the warehouse or in the stacks of jute sacks, prophylactic spraying with Actellic 25(R) (ICI, England) is recommended.

The use of methyl bromide, picrin, ethylene dibromide : methyl bromide (1:1) besides ammonia and sulphur dioxide for control of grain fungi have been reported (Majumder et al., 1955; Ragunathan et al., 1969; Tsuranta and Ishirava, 1966). As with insects, the susceptibility of resistance of fungi to a fumigant can be attributed to three main factors: the stage of the fungal life cycle, its location in the grain and environmental fumigation method. For example, Yanai et al. (1964) showed that mycelia of *Penicillium islandicum* was more susceptible to ethylene oxide than the spore. Studies with several *Aspergillus* spp. showed that spores are more susceptible than conidiophores to methyl bromide. In *Aspergillus ochraceus*, sclerotia required 32 mg/l, double the amount required to control conidia. On the other hand, *Rhizoctonia solani* producing sclerotia could not be controlled with pentachloronitrobenzene (PNCB) (Sharia and Sinclair, 1965). Although ethylene dibromide and chloropicrin killed spores of *Aspergillus flavus* Link and *A. niger* LD_{95} at 10 $mg.l^{-1}$ in laboratory tests, the same fumigant could not control all the fungi if it is present inside the grain (Narasimhan and Rangaswamy, 1968). Other attempts to use chemical preservation for inhibiting fungal growth on stored grain has been reviewed by Majumder et al. (1963). Non of the treatments were found to be completely successful in preventing fungal growth in grains. Indeed, Odamtten (1982) showed that fumigation did not kill fungi when used a prophylactic spray in the warehouse. Mycotoxins seem unaffected by common chemical fumigants (Brekke et al., 1978) or preservatives (Tuite, 1979) except for ammonia which is used to destroy aflatoxin (Brekke et al., 1975; Anon, 1977). Fumigation also poses a health hazard to workers in food processing factory through the formation of chlorohydrins (LD_{50} equal to 0.07 g/kg test animal body weight) a possible direct toxin effect (Van Kooij, 1981).

In view of this toxic effect of fumigants, the Environmental Protection Agency (EPA) of the USA has announced the cancellation and phase-out of all major pesticide uses of ethylene dibromide (EDB). The use of EDB for quarantine fumigation of fruits, vegetables, cereals, etc. phased out on 1st September 1984. The EPA had earlier on ordered the immediate emergency suspension of EDB as soil fumigant for agricultural crops on 30th September 1983 (Anon, 1983). This action underscores the increasing cost of chemicals for third world countries and the outcry against continuous use of chemical sterilants owing to their residue problem. Public Health Authorities throughout the world have become stringent in their regulations to curtail chemical burden in human food. The application of ionization radiation as a process method offers a better alternative to chemical insecticide and fumigants because application of ionizing radiation leaves no chemical residue and no insect or fungus has been found to develop immunity to radiation. Application of gamma irradiation in combination with mild heat treatment to control growth and toxin production of *A. flavus* in maize forms the basis of the investigations as outlined below. A suitable packaging material for pre and post irradiation storage of grains was investigated.

Aim and sequence of the investigations

Insufficient work has been carried out to examine Ghanaian mouldy maize for the presence of potential mycotoxin-producing fungal species although there have been sporadic reports of acute food poisoning (and sometimes death) arising from ingestion of maize contaminated by mixtures of growing fungi. The situation is such that any grain lots with an objectionable aesthetic look attributable to fungal discolouration is inadvertently passed on for use as ingredient in poultry and livestock feed. The detrimental consequence is that mycotoxin contamination of meat, egg and milk at farm is imminent after feeding animals with contaminated rations. Transmission of aflatoxins and Ochratoxin A from contaminated rations have been demonstrated in dairy cattle, pigs and poulty (Elling et al., 1975; Krogh, 1977; Rodricks and Stoloff, 1977). The water activity a_w and temperature conditions under which some toxigenic fungi produce potent mycotoxins (aflatoxins, Ochratoxin A, patulin, penicillic acid) have been exhaustively studied by Northolt (1979), in maize by Gough and Bateman, 1977; Koehler, 1938; Lopez and Christensen, 1967; Ciegler and Kurtzman, 1970; Ciegler, 1972; Kurtzman and Ciegler, 1970). These conditions are akin to the environmental conditions that occur in our tropical region and is responsible for the continuous survival of fungal spores in food and the attendant losses in nutritive and biochemical values of foods.

The objective in Article 1 was to update the storage fungi associated with maize grains kept under ambient Ghanaian tropical conditions for six months and to acquire information on the infection patterns of individual fungi. Once formed the mycotoxin is imparted to the foodstuff and although the fungus may not be isolated after a certain length of time, the mycotoxin, being stable may pass industrial processing of the produce without large reduction and enter into consumer products. Secondly, the particular toxigenic fungi should be identified if the extent of the problem is to be appreciated and effective control measure is to be designed to prevent growth and toxin formation by combination treatment of heat and gamma irradiation. Sufficient information on contaminating species of fungi and their infection pattern within six months storage was acquired. Toxin-producing species. *Aspergillus flavus* Link (aflatoxin B_1 and B_2), *A. ochraceus* Wilhelm (Ochratoxin A and penicillic acid), *Fusarium moniliforme* Sheldon (Moniliformin), *Paecilomyces varioti*, *Penicillium expansum* (Patulin) and several other *Penicillium sp.* were encountered. This information was used in the next experiments.

A. flavus was chosen as the test organisms to ascertain the efficacy of the combination treatment of heat and gamma irradiation in preventing fungal growth and mycotoxin formation for the following reasons: (i) aflatoxins frequently occur in maize and other food grain products, cereal grains, whole wheat, rye bread, cheese, meat, groundnut and nut products, fruit juice, copra and cotton seed and cocoa beans; (ii) its importance as the most potent and carcinogenic mycotoxin of human and animal health importance and (iii) *A. flavus* as the most ubiquitous fungus frequently encountered in stored food grain is also the most radioresistant fungal species. Previous studies by Odamtten (1979) with maize and Amoake-Atta et al. (1981) on cocoa beans showed that a complete control of *A. flavus* could not be obtained with a dose of 5.0 KGy of gamma radiation because high environmental storage R.H. ($\geq$80%) increased the radioresistance of the spores of *A. flavus* by making more free water available to spores for resumed growth after the radiation treatment. Therefore, any effective control method for *A. flavus* growth and of toxin formation could be extended to cater for the other radiosensitive fungi infecting maize grains in storage.

In Article 2 a new apparatus is described which enables the application of either dry heat (60 ^{o}C for 30 min) administered under low humidity ($\leq$45% R.H.) conditions or moist heat under high ($\geq$85% R.H.) ambient conditions to spores of fungi in a heat-treatment chamber prior to gamma irradiation. This combination treatment gives a synergistic effect by allowing greater inactivation of spores at a lower dose of radiation, or heat applied alone. Results of this feasibility study was used in the subsequent investigation.

In Article 3, 'in vitro' studies were carried out to investigate the fungicidal and synergistic effect of the combination treatment of heat and irradiation on the survival of wet and dry conidia of *A. flavus* Link NRRL 5906. Results presented indicate that inactivation of growth of *A. flavus* spores by the combination treatment is feasible.

Articles 4 and 5 demonstrate, in static liquid culture, maize meal broth that both growth and aflatoxin B_1 and B_2 formation by *A. flavus* can be attenuated by combination treatment of moist heat (60 ^{o}C for 30 min) applied under high humidity ($\geq$85% R.H.) conditions in combination with 4.0 KGy of gamma irradiation.

Article 5 presents results illustrating the effect of inoculum size of *A. flavus* spores on aflatoxin production. Aflatoxin levels formed in cultures tend to be inversely related to the inoculum size of *A. flavus* used in inoculating flasks. Results helped in elucidating why some workers obtained enhanced aflatoxin formation via gamma irradiation. It also provided pertinent data on how aflatoxin B_1 levels produced by *A. flavus* in stored grains might be related to the inoculum size with which grain is infected.

Some workers are skeptical about the reproducibility of 'in vitro' results 'in vivo' on the product itself, so far as mycotoxin production is concerned. Article 6 demonstrates positive prevention of aflatoxin B formation by *A. flavus* artificially inoculated onto the product maize and then subjected to the same combination treatment that attenuated the production of aflatoxin B_1 and B_2 in articles 4 and 5.

Apart from preventing recontamination of irradiated cereals (maize, sorghum, millet, rice, etc.), a suitable packaging material should not harbour and support 'de novo' growth of fungal spores when the average tropical R.H ($\geq$80%) is attained and thus act as a springboard for infecting enclosed food grains. Owing to the robust handling of packed cereal grains e.g. maize in transit and in the warehouse, it would be ideal to have a packaging material with high tensile strength which would be able to withstand such rough handlings. Investigations in Articles 7, 8 and 9 were designed to provide pertinent information on the mycological, radiation response and physical parameters (tensile strength) respectively that would assist in the selection of the most suitable of two packaging material compared in these series of studies namely the incumbent and conventional jute sack and the new introduction: synthetic woven polypropylene sack.

In the last experimental sections of the thesis the questions of (i) whether woven polypropylene sacks will also be suitable for storage of other food products e.g. groundnut (*Arachis hypogene* L.) bambara beans (*Voandzeae subterranea*), sorghum (*Sorghum vulgare*), cowpeas (*Vigna unguiculata*) Walp and cocoa beans was investigated. These food products are presently stored in jute sacks with attendant heavy ($\geq$30%) losses and are often kept in the same warehouse. To prevent cross-infestation by insects and cross-contamination with fungi, it would be ideal to use same packaging material for food products with common storage pests and microbial contaminants. If woven polypropylene is more suitable than jute sack even with no irradiation, its protective properties would be augmented by radiation treatment.

Finally, the effect of the combination treatment of heat and gamma irradiation on some physical and chemical properties of maize grains, i.e. sensory evaluation and quality of product is examined.

Findings of Articles 1-9 in the thesis are discussed in the chapter on Discussion in the light of the results obtained and practical implications with regard to safeguarding combined-treated cereal grains from re-contamination during storage, by using the recommended storage sack is highlighted. It is considered that good management practices and clean warehouses would augment the radiation process.

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1. A SURVEY OF THE MYCOFLORA OF MAIZE GRAINS STORED AT 26±3 ^{o}C AND 75±5% R.H.

Abstract

Twenty-six different fungi belonging to twelve genera were isolated and identified on maize (*Zea mays* L.) stored for six months. Nineteen of these are recorded for the first time on maize in Ghana. Members of the genera *Aspergillus* and *Penicillium* were most frequently encountered. *Aspergillus flavus* Link and *Rhizopus oryzae* Went and Prinsen - Geerlings were the most common species isolated at each monthly sampling.

The storage fungi showed four distinct patterns of infection over the entire period of examination. The importance of some of the isolated fungi as potential mycotoxin - producing species is discussed.

Key words: survey, mycoflora, *Zea mays* L., maize.

1.1 Introduction

Maize, an important staple food crop in West Africa, is usually stored over long periods for both human consumption, and as component feed for poultry and livestock. Post-harvest storage losses of the maize grains within West Africa due to insects attack and microbial spoilage is estimated at 30% (1, 40) of annual harvest. The role of storage fungi in quality deterioration of grains although well documented (4, 7, 18) in developed countries, there is limited information regarding these fungi on maize in West Africa and the role such fungi play. The objectives of these investigations are to update the list of storage fungi associated with maize grains and also to acquire information on the infection pattern of these moulds as well as to determine if there are any toxin-producing fungi on maize in Ghana.

1.2 Materials and methods

Maize (*Zea mays* L.) grains stored in jute bags under ambient laboratory conditions (75±5% R.H. and 26±3 ^{o}C) were sampled monthly for six months. At each monthly sampling, 500 g of grains were taken from the top, middle and bottom of the jute bag and pooled together. The mycoflora on the grains was assessed using the method of Tempe (37) and Limonard (19), washing test and solid medium.

1.2.1 Blotter method

A modified method of Tempe (37) and Limonard (19) was employed. Ten grains, surface-sterilized with 2% sodium hypochlorite for 15 min. and ten non-surface sterilized grains were placed on Whatman's filter paper in 9.0 cm Petri dishes. There were 50 replicates (500 grains) for each treatment and the plates were incubated for up to 14 days (14) at 26±3 ^{o}C.

The following monthly quantitative assessments were made for blotter test:

a) the percentage of grains infected with fungi;

b) the percentage of grains infected with a particular fungus species;

c) the total number of fungal colonies appearing on the grains.

1.2.2 Washing method

The 2% sodium hypochlorite solution used for surface sterilization was centrifuged at 2,500 g for 10 min. in an M.S.E. Super Minor Centrifuge. The supernatant was discarded and the residue (with fungal spores) resuspended in 10 ml distilled for microscopic examination and identification using a Reichert Nr. 4253 binocular microscope.

1.2.3 Solid medium method

Ten surface-sterilized and ten non-sterilized grains were placed on Sabouraud's Agar in 9.0 cm Petri dishes without any further treatment. There were 50 replicates (500 grains) for each treatment and the plates were incubated at 26±3 ^{o}C until fungi grew.

Sodium hypochlorite treatment was used with the aim of reducing or removing completely external saprophytes which compete with pathogens.

1.2.4 Estimation of viable fungal colonies

Exactly 50 g of sample was weighed into 100 ml 0.1% Peptone in 250 ml Erlenmeyer flasks and were shaken in a Gallenkamp Model Orbital Shaker (140 rev./min.) for 30 min. From this stock suspension serial dilution method was employed and spores were raised on Sabouraud's Agar.

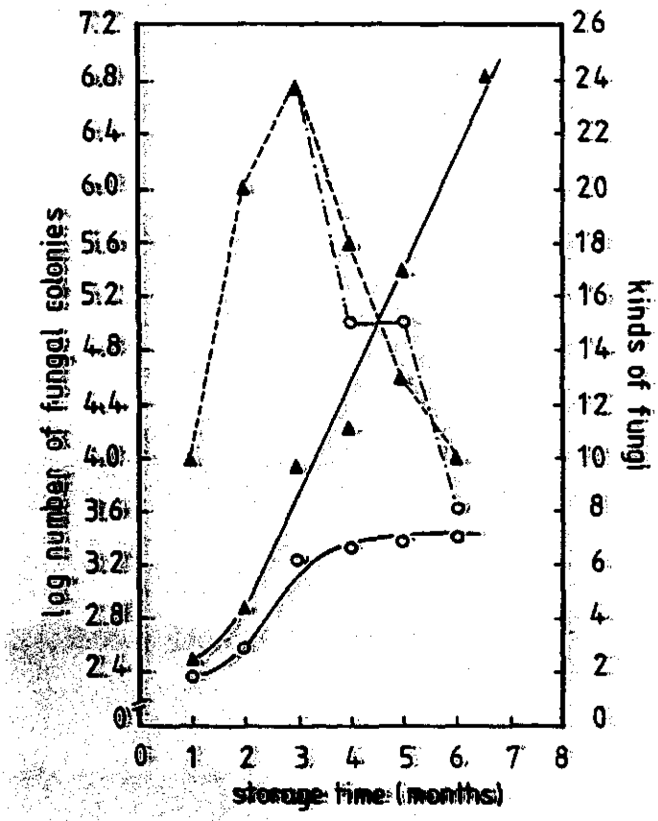

Fig. 1. Fungal growth and kinds of fungi (N$^{\underline{o}}$ of species) recorded on maize grains stored for six months.
Fungal colonies: ▲——▲ Control; o——o Surface-sterilized.
Kinds of fungi: ▲- - -▲ Control; o-·-·-o Surface-sterilized.

1.2.5 Moister content determination

Moisture content of grains at each sampling was determined using the oven dry method. Samples of the grains were ground in Waring Blender and 10 g portions of their flour were dried in an oven for 24 h at 105 °C and then cooled in a desiccator and reweighed.

1.3 Results

There was a rapid increase in fungal colony counts in stored maize grains with time whilst colonies on surface-sterilized grains showed suppressed growth compared to the untreated grains; total fungal colony on the surface-sterilized grains remaining constant after the 4th month (Fig. 1). The kinds of fungi (number of species) isolated showed an initial increase and continued up to the 3rd month (24 different kinds) and thereafter declined (Fig. 1). The same trend was exhibited by fungi appearing on surface-sterilized grains which reached the same peak number of 24 different species on the 3rd month (Fig. 1). The moulds which were recorded on untreated grains were the same as those which appeared on the surface-sterilized grains. The twenty-six different fungi belonging to twelve genera isolated from maize grains in these investigations are listed in Table 1. Seed-borne fungal species recorded for the first time on maize in Ghana were *Aspergillus candidus* Link, *A. flavus* Link, *A. fumigatus* Fresenius, *A. niger* van *Tiechem*, *A. ochraceus* Wilhelm, *A. restictus* G. Smith, *A. temarii* Blochwitz, *A. ustus* (Bainier) Thom & Church, *A. wentii* Wehmer, *Cladosporium herbarum* Link ex Fr., *Neurospora sitophila* Shear & Dodge, *Paecilomyces varioti* Bain, *Penicillium digitatum* Sacc, *Penicillium expansum* Link ex Fries, *P. verrucosum* var. *cyclopium*, *Penicillium sp.*, *Phoma glomerata*, *Rhizoctonia solani* Kuhn, and *Rhizopus oryzae* Went and Prinsen-Geerlings. Members of the genus *Aspergillus* were the most predominant followed by *Penicillium*. *Aspergillus flavus* infection counts were highest at each monthly sampling (Figs. 2 and 3).

Only nine different fungal spores were encountered in the washing test, namely *Aspergillus amstelodami* Mangin T & C, *A. flavus*, *A. janus*, *A. ruber* Spieck & Bremer, *A. sydowi* (Bain & Sart) Thom & Church, *Aspergillus sp.*, *Fusarium sp.*, *Neurospora sitophila* and *Phoma glomerata*. Of these, only *A. flavus*, *N. sitophila* and *P. glomerata* appeared in the blotter test and solid medium method and were thus viable spores. Moulds isolated by the solid medium method were same as those obtained by the blotter method.

Four distinct patterns of infection were exhibited by the storage fungi

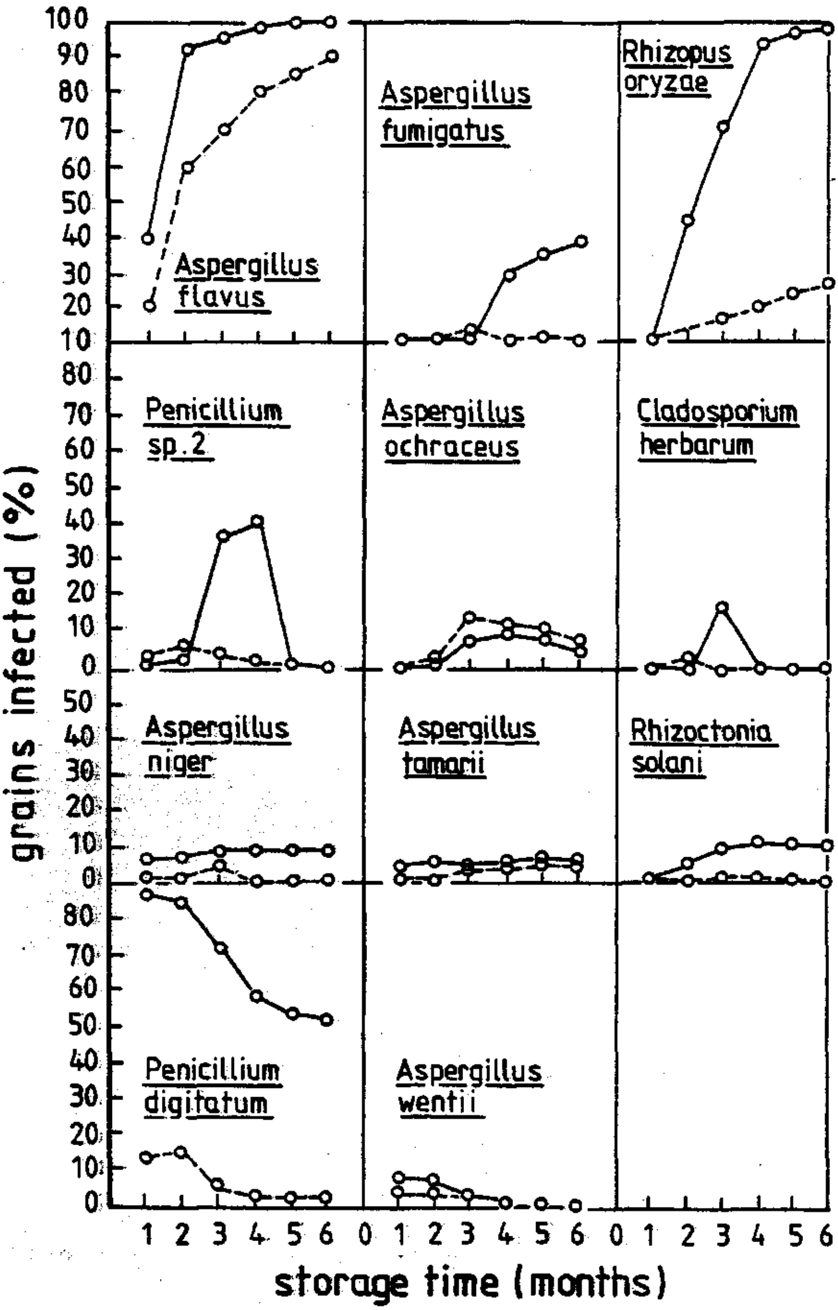

Fig. 2. Selected fungal species identified on maize grains showing four different patterns of infection.
o——o Control; o---o Surface-sterilized.

during the six months period (Fig. 2). The percentage number of grains infected by *A. flavus, A. fumigatus* and *Rhizopus oryzae* increase with storage time whilst the percentage number of grains infected with *Penicillium digitatum* and *A. wentii* decrease within the same period (Fig. 2). In contrast with this, there was an initial increase in percentage of grains infected by *Penicillium expaneum* (*Penicillium sp. 2*), *A. ochraceus* and *Cladosporium herbarum* up to 2-3 months followed by a sharp decline (Fig. 2). Finally, the percentage of grains infected by *A. niger, A. tamarii* and *Rhizoctonia solani* remained fairly constant, after three months storage period up to the sixth month (Fig. 2). Selected six fungal species that showed the first two patterns of infection illustrated in Fig 2 is presented in Fig. 3. The fungi *A. wentii, C. herbarum* and *Fusarium moniliforme* could not be isolated after four months from the non-sterilized maize grains (Figs 2 and 3).

The fungus *A. flavus* infected 90-100% of the surface-sterilized grains after three months while *A. ochraceus* and *F. moniliforme* were present in about 6% of the grains (Figs 2 and 3).

The moisture content of the maize grains varied from an initial 12.1% to 15.1% in six months (Table 2).

1.4 Discussion

Small amounts of fungal spores present on the surface of the grains or as dormant mycelia under the pericarp grow fast under favourable conditions. The number of fungi developing on surface-sterilized grains was reduced as compared to the untreated grains (Fig. 1) because sodium hypochlorite treatment reduced or removed completely external saprophytes which compete with the pathogens. The moulds which grew on the untreated maize were the same as those obtained on the pre-treated ones and were thus seed-borne. This observation confirms that of Lichwardt and co-workers (17) for maize.

Danquah (9) isolated twenty-two different seed-borne fungi on maize in Ghana. In these investigations, nineteen new species have been identified as seed-borne fungi on maize in Ghana (Table 1). This extends to forty-one, the number of seed-borne fungi recorded on maize in Ghana. The fungi encountered in this paper belonged to twelve genera. The new genera added are *Cladosporium, Neurospora, Pascilomyces* and *Rhizoctonia*.

Broadbent and co-workers (2) provided an extensive list of fungi associated with maize in Nigeria. Later, other workers (28, 29) added eleven different fungi to this list. Koubasher et al. (22) isolated and identified thirty-

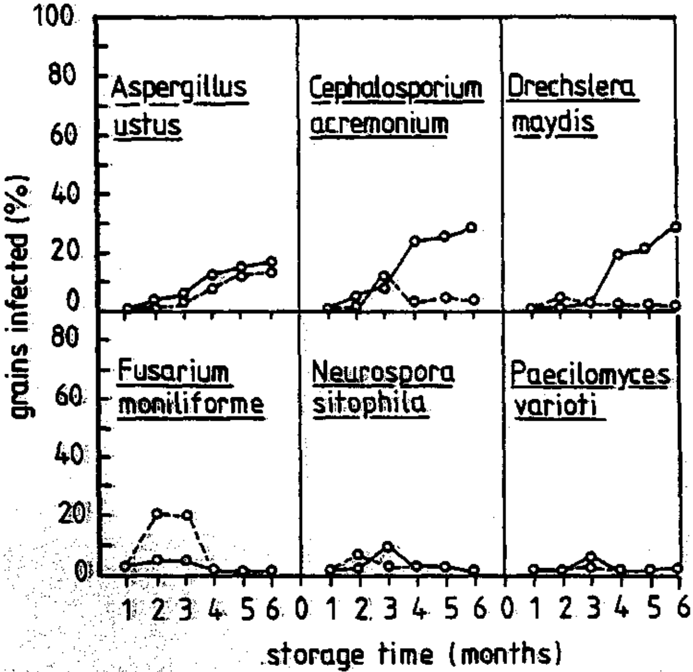

Fig. 3. Selected fungal species showing the first two patterns of infection illustrated in Fig. 2.
o——o Control; o---o Surface-sterilized.

four species of fungi belonging to thirteen genera on stored maize in Egypt. Members of the genus *Aspergillus* were the most predominant followed by *Penicillium* (22). Results obtained in this work (Table 1) agree with this. The method of isolation may influence the species of fungi encountered although in these investigations no difference was found between kinds (species) of fungi isolated by the blotter test and on Sabouraud's Agar. The washing test revealed spores of nine fungi which were loosely borne on the grains. Out of these only *A. flavus, N. sitophila* and *P. glomerata* appeared in the blotter test and solid medium method. It is evident that although the washing test gives some information of fungal spores associated with grain lots conclusions cannot be made from the results of a washing test because spores encountered may not be viable (25).

The quality of grains used is dependent on its wholeness as well as the presence and dominance of certain microorganisms which may be detrimental to its quality (24). The kinds of fungi present on any grain stock woild also depend on the geographical location, prevailing weather conditions in the locality and the post-harvest storage conditions. Four different infection patterns have been observed in the seed-borne fungi on maize in Ghana. This suggests that the mycoflora that would be present in any grain stock at a particular time would depend on the length of the storage period, for indeed, *Aspergillus wentii, C. herbarum* and *F. moniliforma* could not be isolated from the untreated grains, after four months (Figs 2 & 3). The alteration in biochemical qualities of the basal medium (maize) owing to the metabolic activities of the mycoflora probably become selective favouring the growth of some and deleterious to others. It is indeed documented (43) that no growth and aflatoxins formation by *A. flavus* were detected in maize grains when *A. niger* NRRL 6411 or *Trichoderma viride* NRRL 6418 were paired with *A. flavus* in inoculated grains.

For each of the common species of storage fungi, there is a minimum moisture content in grains below which the fungus cannot grow. These minimum moisture contents have been determinated for most of the common storage fungi growing on starchy cereal grains (7). Davey and Elcoate (10) put the safe moisture content for storage of maize at about 13% whilst Lutey and Christensen (20) suggested that viable field and storage fungi may be generally reduced or eliminated from grain lots stored at moisture content 12-14% and a temperature of 20-30 ^{o}C without serious deterioration to grains.

This partly explains why the moisture content of the grains rising above the stipulated safe level, permitted heavy invasion and growth of storage fungi

Table 1. Seed-borne fungi recorded on maize in this investigations under ambient (75±5% R.H. and 26.0 ± 3 °C) conditions.

**Aspergillus candidus* Link	*Drechslera maydis* (Nisikado) Subraim & Jain
**A. flavus* Link	*Fusarium moniliforme* Sheldon
**A. fumigatus* Fresenius	*Fusarium* sp.
**A. niger* van Tieghem	**Neurospora sitophila* Sheard Dodge
**A. ochraceus* Wilhelm	**Paecilomyces varioti* Bain
**A. restrictus* G. Smith	**Penicillium digitatum* Sacc.
**A. tamarii* Blochwitz	**P. expansum* Link ex Fr.
**A. ustus* Wehmer	**P. verrucosum* var. *cyclopium*
**A. wentii* Wehmer	*Penicillium* sp. 4
Aspergillus sp.	**Penicillium* sp. 5
Cephalosporium acremonium Corda	**Phoma glomerata*
**Cladosporium herbarum* Link ex Fr.	**Rhizoctonia solani* Kuhn
Curvularia lunata (Wakker) Boedjin	**Rhizopus oryzae* Went and Prinsen-Geerlings.

* New record on maize in Ghana; unmarked: recorded by Danquah (9).

Table 2. Comparative moisture content of maize grains during six months storage period at 26±3 °C and 75±5% R.H.

Storage period (months)	% Moisture content (mean ± S.E.)
1	12.1 ± 0.1
2	14.3 ± 0.1
3	14.6 ± 0.2
4	14.7 ± 0.3
5	14.6 ± 0.3
6	15.1 ± 0.2

(Table 2). Noubasher and co-workers (22), indeed presented evidence to show that the highest population of fungi was obtained from grains with a moisture content of 15.2% stored at 30 °C for four months. Data from Fig. 1 and Table 2 support their observation.

In this study, potential toxin-producing fungi, namely *A. flavus*, *A. ochraceus*, *F. moniliforme*, *Paecilomyces varieti* and *Penicillium expansum* were encountered. Much pertinent literature exists on mycotoxicoses in animals, including man, caused chiefly by *A. flavus* (3, 13, 21, 32, etc.), *A. ochraceus* (6, 39, 41, 42), *Fusarium* (16, 26), *Penicillium* (3, 30, 42, 44), and *Paecilomyces varioti* (8).

The mycotoxins aflatoxin B_1 and B_2 are produced in maize (11, 16, 28, 33, etc.) by *A. flavus*. Brooke and White (3) reported that it was not necessary for the product to be thoroughly and heavily overgrown by *A. flavus* before toxin will be present. If 3-5% of the grains are invaded, aflatoxins will be detected. About 40% of the maize grains in this paper were invaded by *A. flavus* after one month storage and the number increased to 95% two months later (Fig. 2).

Some strains of *A. ochraceus* produce ochratoxin (38, 41). Isolates capable of producing ochratoxin are common in black pepper and maize (5, 6), dry fish (39), rice (23) and peanuts (12). Ochratoxin A is a potent nephrotoxin in experimental chicks (15), rats (31, 34), dogs (35) and swine (36). Ochratoxin A based on 50% lethal dose determinations and minimal growth - inhibitory concentration, is the most potent mycotoxin studied in chickens to dat (15). An 8-15% infection of grains by this fungus between 2-6 months (Fig. 2) is sufficient to warrant concern.

Different kinds of extremely potent metabolites are produced by species of *Fusarium* (16, 27). The fungus *F. moniliforme* isolated in this work produced toxin under laboratoy conditions but it is not known whether it does so in nature. Presently, there is no information regarding the concentration of the toxin that would render maize toxic for human and animal consumption. It would be interesting to investigate this in order to ascertain whether the population of *F. moniliforme* present in 20% of the grains after 2-3 months storage was sufficient to produce enough toxin, since *F. moniliforme* could not be isolated after four months.

The percentage of grains invaded by *Paecilomyces varioti* was highest (8%) on the third month and thereafter declined (Fig. 3). According to Covency and co-workers (8) *P. varioti* does produce a potent toxin.

Eighty-three out of 196 *Penicillium sp.* screened for possible toxic metabolites caused death, in less than seven days, to animals to which these were fed (3). Five different *Penicillium sp.* have been recorded on stored maize and is reported in this paper. Their potential for producing toxic metabolites remain to be assessed.

Because of the presence of toxin-producing fungi on stored maize grains, indiscriminate use of contaminated grains for poultry feed and human consumption should be discouraged or discontinued. Improved quality of food is as important as increased quantity. Hence, programmes to control fungal deterioration of maize grains in storage should aim at preventing growth of fungi and thus the production of toxic mycotoxins.

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2. STUDIES ON THE TECHNOLOGICAL FEASIBILITY OF THE APPLICATION OF DRY OR MOIST HEAT TO GRAINS AND GRAIN PRODUCTS PRIOR TO GAMMA IRRADIATION

G.T. Odamtten, V. Appiah, and D.I. Langerak

SUMMARY

A simple apparatus has been designed that enabled us to heat maize grains and grain products sustained at 60 °C for at least half an hour at precise humidities, low (≤ 45% R.H.) or high (≥ 85%) during the heat treatment. The process is more efficient and temperature and humidity required are reached within 5 min if the grains and grain products are heated in open containers. It was advisable to allow the chamber to reach 'equilibrium' (required humidity and temperature) before introducing the product into the climate chamber. The practical implications of these findings are discussed bearing in mind that the heat treatment at high humidity does not significantly increase the m.c of the grains after the treatment.

INTRODUCTION

Heat treatment of food is a well established and accepted method of processing food for consumption whilst ionization radiation has only recently been proven as a method of killing or reducing microbial load of stored products. In some instances, such as cocoa (AMOAKO-ATTA et al., 1980), and maize (ODAMTTEN, 1980), irradiation alone up to 5.0 kGy could not give complete control of pathogenic moulds because the fungistatic effect of the radiation was dependent on the storage humidity. Radiation treatment combined with heat treatment (KISS & CLARKE, 1969; ROY et al., 1972; SOMMER et al., 1972; LANGERAK & CAÑET-PRADES, 1979 etc.) was more effective than radiation alone.

According to WEBB and co-workers (1969) and SOMMER et al. (1972), the moisture content of spores or conidia prior to irradiation, as well as in the developmental stage of the mycelia, affect the radiation resistance of fungi. In the opinion of NYERGES-ROGRÜN (1973) conidia irradiated in the wet state proved to be more radiation-sensitive than those treated in the dry state. It is the aim of the treatment with high (> 85% R.H.) humidity during heat treatment of grains to enhance the killing effect of heat on the pathogenic moulds.

The question of the possibility of providing high humidity (> 85% R.H.) conditions as compared to low humidity (< 45% R.H.) conditions during the heat treatment spured us on to examine the use of a simple apparatus that would enable us monitor the heat penetration into the product and ambient humidity in the chamber during the heat treatment.

MATERIALS AND METHODS

The heat treatment was initial carried out in a large climate room with polystyrene lining (320 m long x 160 m wide x 207 m high) at the Foundation Institute for Atomic Sciences in Agriculture - ITAL, Wageningen, where an evaporator (Defensor 505) provided the requisite ambient humidity and the inbuilt heater (Contardo Model PAI 2, Milano, Italy) in the wall of the room provided the desired temperature of 60 °C. The thermocouples were inserted into small holes (Ø/mm) bored at the radicle end of the grains and in the case of the apples 2 mm below the peel of the apples. Apple was used to find out if the process will be applicable to fruits. The temperature was recorded as the potential difference between the cold (ice) point and hot point (internal portion of grains) on a potentiometer 5 criber caliberated on temperature scale (0 - 100 °C). The humidity was recorded by a digital Psychrometer (PTM). The samples were placed in a polystyrene container (25 x 20 x 20 cm) and opened just before experiment commenced. The temperature was regularly recorded (5 min intervals) for 1 h and then after 1½ h.

The volume of the large climate room did not allow, temperature and humidity to rise above 55 °C and 75% R.H. respectively. Therefore, a smaller chamber, an incubator (Marius, Utrecht, The Netherlands), was resorted to using the same ancillary apparatus as in the larger climate room.

Moisture content determination

Two ten (10 g) gram portions of the flour of maize were kept at 105 °C for 24 h or until constant weight was obtained as suggested by AOAC (1960).

RESULTS AND DISCUSSION

When low humidity (< 45% R.H.) was provided during heating, the grains attained a temperature of 53 °C, slightly inferior to that recorded by the thermistor probe (PT 100) which measured the ambient temperature of 55 °C. The maximum temperature of 55 °C was attained after about 50 min heating period (Fig. 1a). In the case of apples, penetration of heat was poor and a temperature difference of about 15 °C between the PT 100 reading and the thermocouple reading was obtained (Fig. 1b). It was clear that this mode of heat treatment was not suitable at least for apples since the heat penetration was well inferior to what obtained in the maize grains. Furthermore, there was browning of the skin of the apples thereafter.

When humidity provided during heating was increased to 74.3% R.H., penetration of heat through the woven polypropylene bags (a and b) was about 25 °C and 15 °C respectively lower than that recorded by the thermistor probe, PT 100. The ambient temperature of 56 °C was never attained by the grains in the bag even after 2 hours heating (Fig. 2a). In contrast to this, grains heated in open shallow trays (c and d) attained the required temperature about 15 min and thereafter remained constant. Treatment of apples with higher humidity proved impracticable (Fig. 2b). It was evident that to obtain good heat penetration during heat treatment, the grains should be heated in open containers before putting in bags for irradiation. This will permit maximum penetration, under high humidity in less than 15 min. In practice, a higher humidity (> 85% R.H.) and higher temperature (60 °C for 30 min) will be required. It was then worthwhile to decrease the volume of the climate chamber to be used and thus improve upon the humidity and temperature distribution to ensure maximum efficiency. Thus a smaller simulated climate chamber was designed, using the same apparatus as in the bigger room but instead using an incubator as the climate chamber. Plates 1, 2, 3 illustrates the external and internal arrangement of the apparatus. The heat treatment was carried out with and without putting

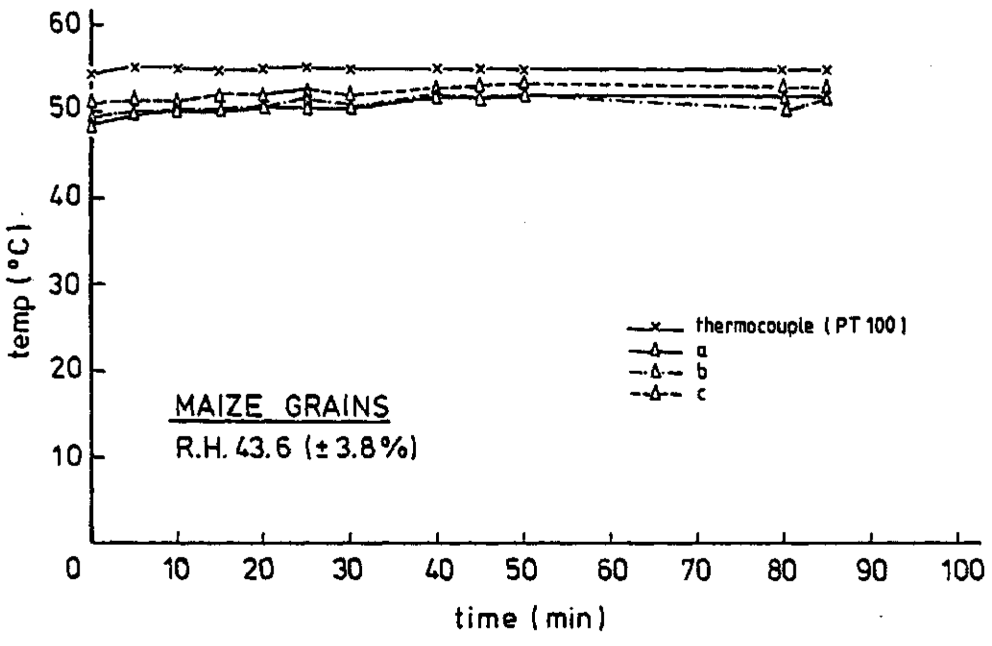

FIG. 1a. Graph showing temperature measurements in maize grain during heat treatment at low humidity (43.6 ± 3.8%) in large climate chamber.

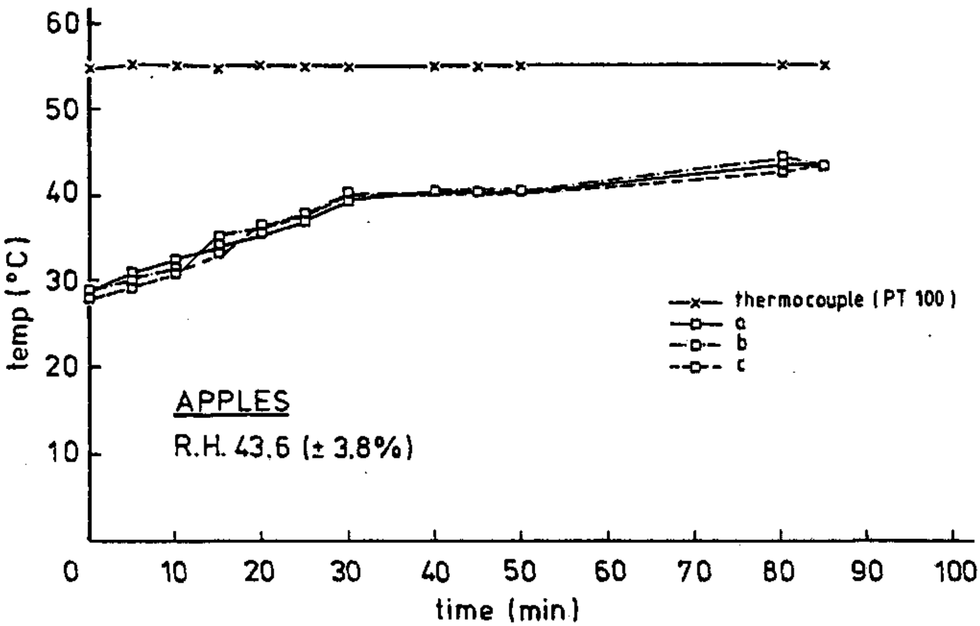

FIG. 1b. Graph showing temperature measurements in apples during heat treatment at low humidity (43.6 ± 3.8%) in large climate chamber.

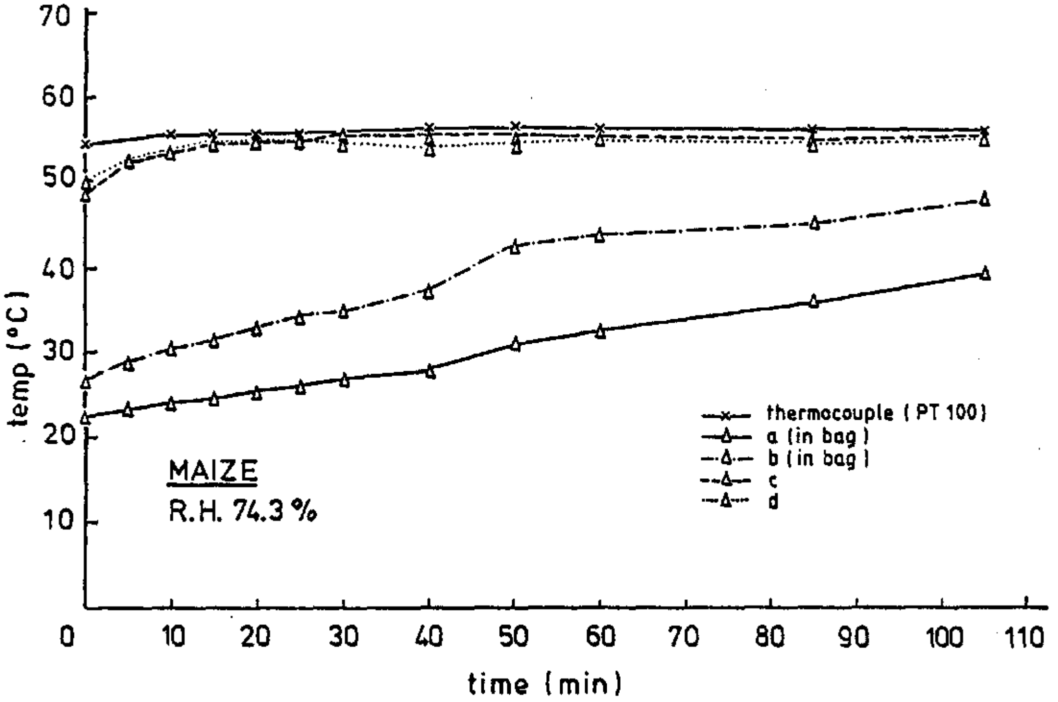

FIG. 2a. Graph showing temperature measurements in maize grains during heat treatment at high humidity (74.3%) in large climate chamber (Note the lag in temperature rise in grains that were treated in bags a and b).

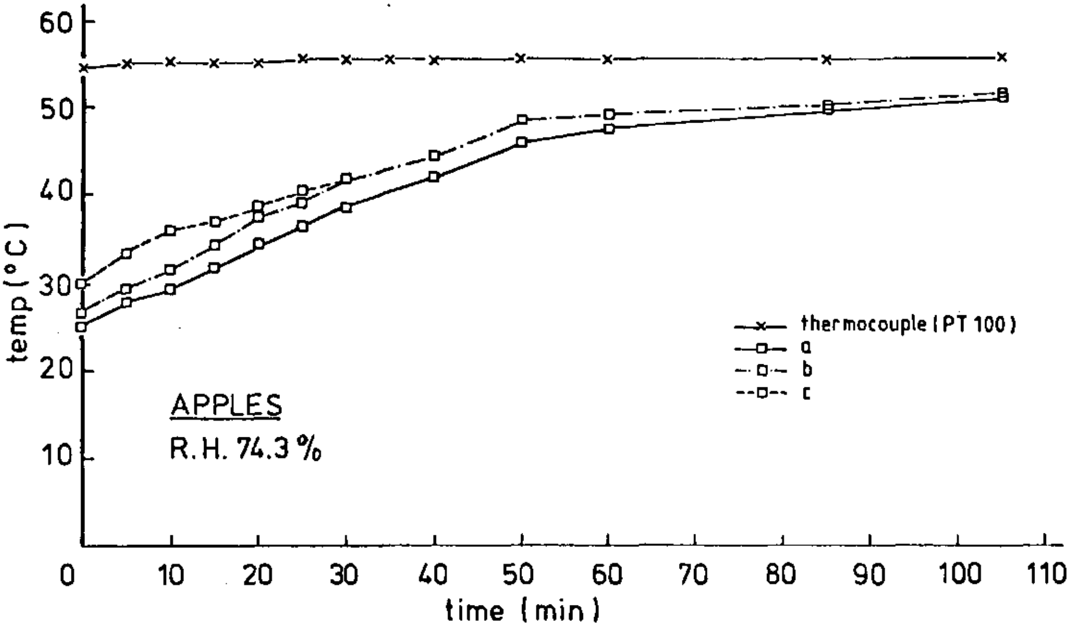

FIG. 2b. Graph showing temperature measurements in apples during same heating period and humidity as indicated in FIG. 2a. in large climate chamber.

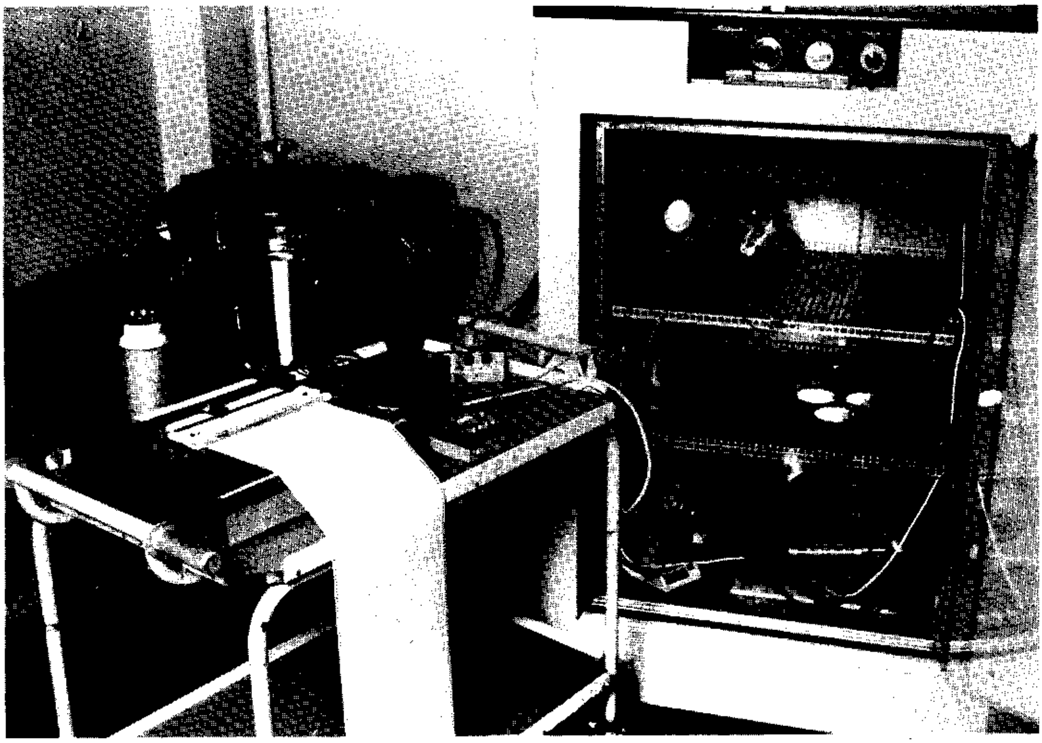

Plate 1. Photograph of the external and internal arrangement of the heat treatment chamber employed in these investigation.

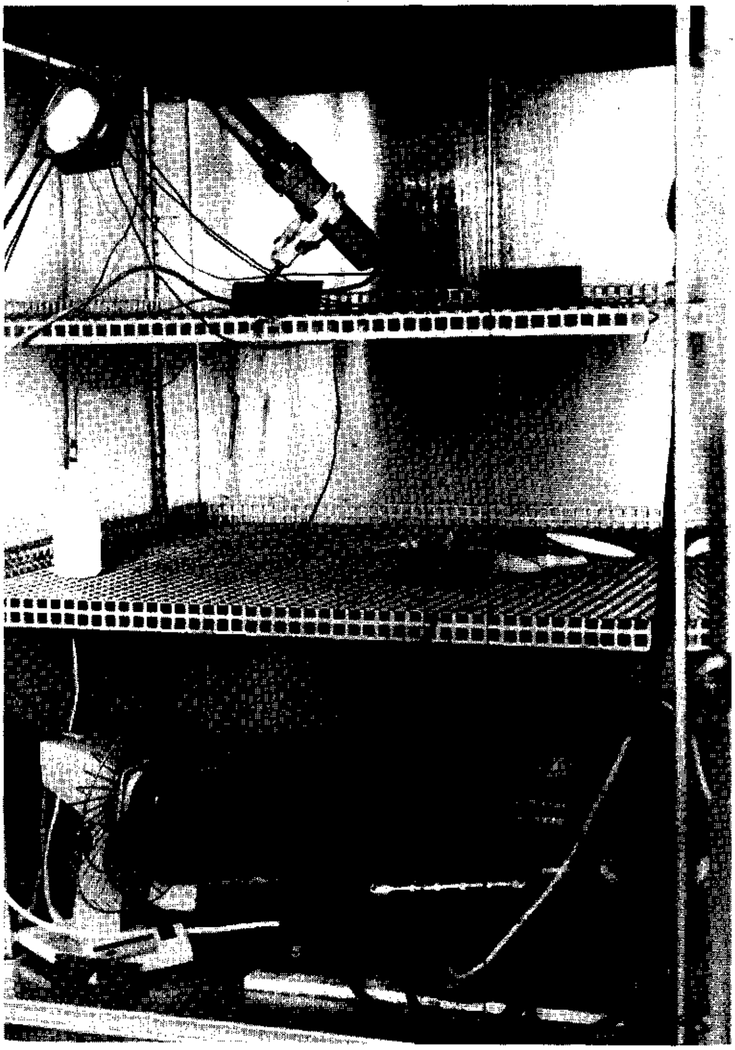

Plate 2. Close-up photograph of the internal portion of the heat-treatment chamber.

1. Hanging mercury thermometer.
2. A TPM Digital Phychrometer which records the ambient humidity and temperature.
3. Petri dishes with lids eccentrically placed on the lid.
4. An eletric fan which together with another smaller one (at top left corner) provides a uniform conventional current of heat and humidity.
5. Control knob for the Defensor 505 Evaporator.
6. A heater to boost the heat provided by the incubator.

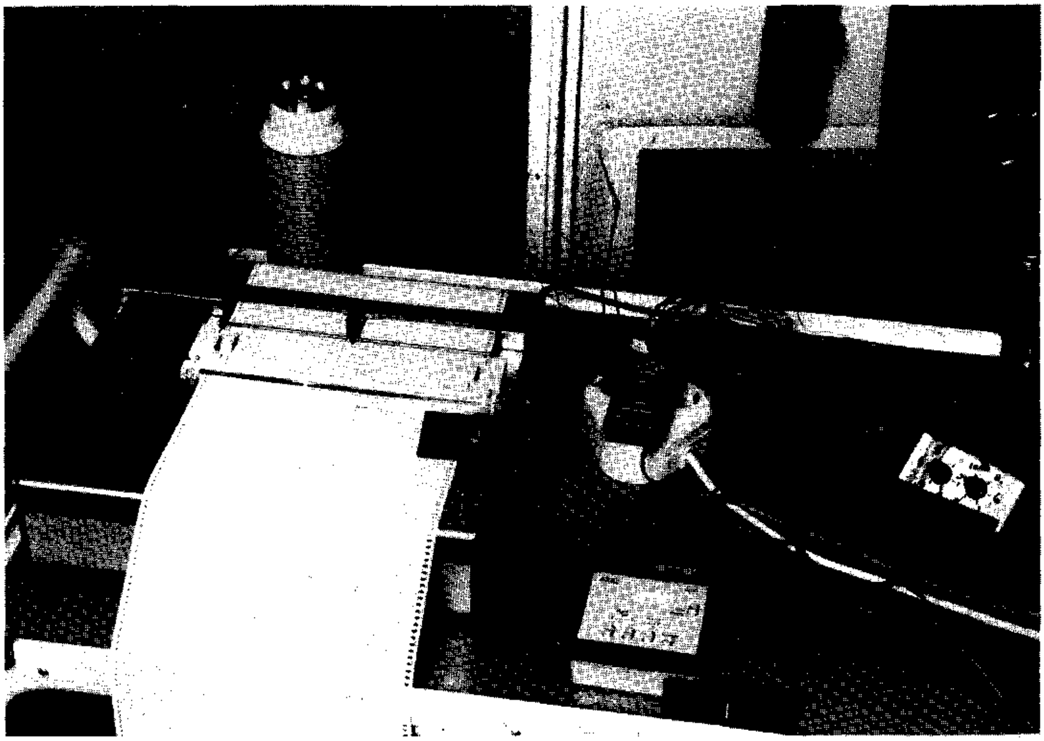

Plate 3. Close-up of the external arrangement of the heat-treatment apparatus.

1. The control Knob for the PTM Phychrometer
2. Control knob for the thermocouples
3. The Potentiometer scriber which converts the "current" from the thermocouples to temperature and record; this is recorded on the chart.

TABLE 1.

Comparative moisture contents of maize grains after heat treatment at high (> 85% R.H.) and low humidity (< 45% R.H.).

Temperature (^{o}C) and humidity (%) during heat treatment	Moisture content of grains (Mean $\pm$ S.D.)
20 L	12.6 $\pm$ 0.4
20 H	12.7 $\pm$ 0.1
60 L	12.9 $\pm$ 0.4
60 H	12.9 $\pm$ 0.1

L - Low humidity (< 45% R.H.)

H - High humidity (> 85% R.H.)

on the incubator heating system and ventilator (Electric fan). The fan improved the ventilation and provided a uniform convectional current of heat and humidity within the chamber. Results presented in Fig. 3 with a heating humidity of 77.3% R.H. showed that it required one hour before the grains in open tray 'a' attained the required 60 °C whilst thermocouple 2 in tray b was below 60 °C. The incubator and ventilator were off in this instance. Putting on the incubator heating system to 60 °C and the heater at the base as well as the fan to improve the convectional current in the chamber permitted a uniform temperature and humidity distribution. A maximum temperature of 60 °C and humidity (high > 85%; low < 45% R.H.) was attained in 5 min after introducing the product into the chamber (Fig. 4). The incubator was prior to this, allowed to 'equilibrate' to attain a temperature of 60 °C and required humidity before putting in the maize grains.

Happily the process did not appreciably increase the moisture content of the maize grains after the heat treatment at high humidity (> 85% R.H.) (Table 1), thus settling the fears that the process might increase the moisture content of the grains to the detriment of the grains during storage thereafter. The primary objective of application of high humidity during heating is to augment the killing effect of heat on mould spores since this provides increased penetration of heat on target organisms. The increased moisture of spores during the heating at high humidity (> 85% R.H.) renders the spores more radiation-sensitive and the subsequent irradiation of the grains should be carried out not more than 30 min after the heat treatment in order not to lose the synergistic effect of the combination treatment.

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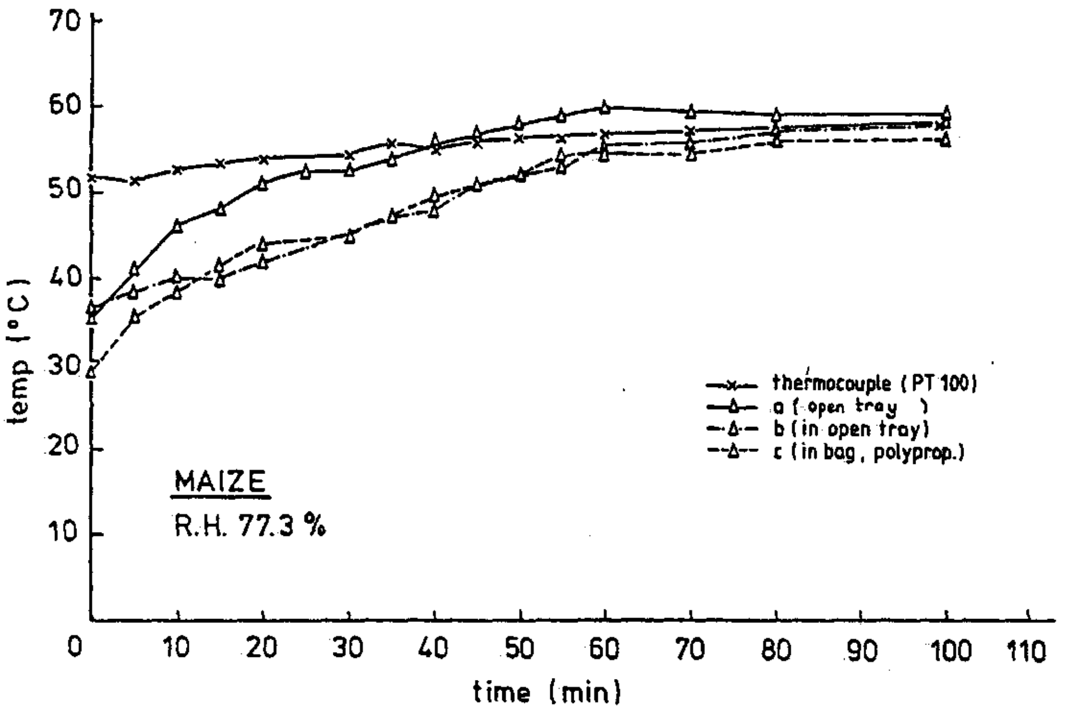

FIG. 3. Graph showing temperature measurments in maize grains during heat treatment at high humidity (77.3% R.H.) in the smaller incubator without additional ventilation and heating.

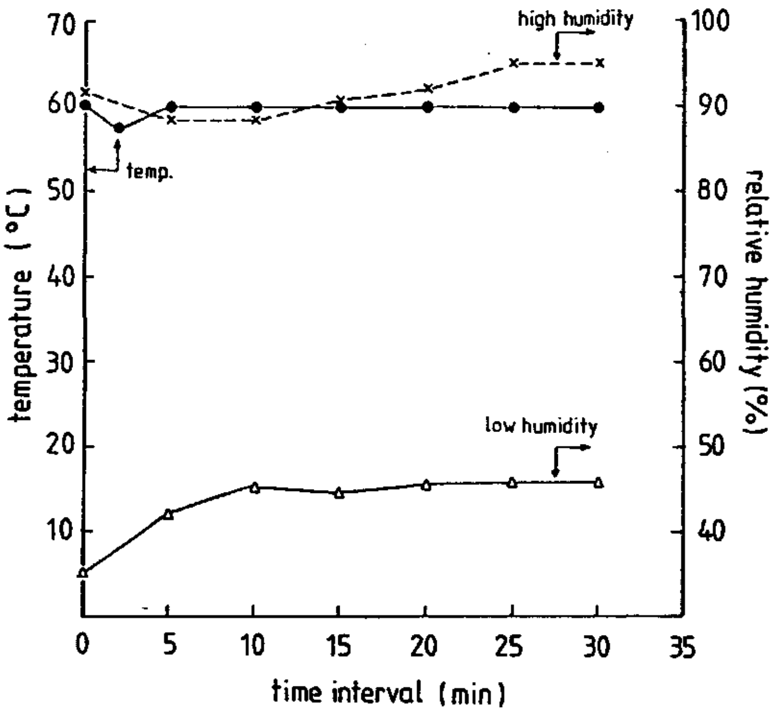

FIG. 4. Graph showing temperature and humidity measurements during heat treatment in the chamber when adequate heat and ventilation (convectional current) was provided.

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3. IN VITRO STUDIES ON THE EFFECT OF THE COMBINATION TREATMENT OF HEAT AND IRRADIATION ON SPORES OF *ASPERGILLUS FLAVUS* LINK NRRL 5906

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Studies have been carried out to investigate the effect of the combination treatment of heat and subsequent gamma irradiation on the survival of conidia of *A. flavus* Link NRRL 5906. The mould spores were heat-treated either in aqueous suspension (containing 0.2% Tween-80) or harvested dry and heat-treated by hot air in a climate chamber at low humidity (≤45% R.H.) or high humidity (≥85% R.H.). Heat treatments of the aqueous suspensions were performed at 20, 40, 45, 48, 50, 52, 53, 55, 58, 59 and 60 °C for 2.5, 5.0 and 10 minutes, respectively. Hot-air treatment of dry-harvested spores was done for 30 minutes at 60 °C. Suspensions were given 0.25, 0.50, 0.70, 1.0 and 1.5 kGy of gamma radiation, while dry harvested spores were exposed to 3.5 and 4.0 kGy. Irradiation was carried out not later than 30 min after heat treatment.

In aqueous suspension the adverse effect of heating on the spores generally started after being at the temperature of 50 °C or higher for at least 2.5 min. Heating for at least 5 min at 59 °C inactivated the moistened spores completely (at least 5 log cycles reduction in the viable count on maize meal agar). When heating of the suspensions was combined with a subsequent irradiation treatment, a synergistic effect was observed at a temperature above 50 °C and the synergism increased with increasing heat damage (increasing the heating time and/or temperature).

Heating of dry conidia at 60 °C for 30 min at high humidity (≥85% R.H.) reduced their viable count by 3 log cycles, whilst a parallel treatment at low humidity (≤45% R.H.) did not appreciably reduce the viable mould spores. However, when the spores were stored at 80% R.H. for 4 days before plating, a considerable fraction recovered from the heat damage. Radiation treatment was more effective after exposing the spores to hot air with high humidity (≥85% R.H.) before irradiation than when low humidity (≤45% R.H.) was applied. The practical implications of these findings are discussed.

Keywords: Combined treatment, irradiation of spores, survival of *A. flavus*

The contamination of food consumed by humans and animals alike by fungi has raised much concern, particularly those fungi which produce potent mycotoxins (Enomoto & Saito, 1972; Austwick, 1975; Tuinstra et al., 1975; Smith et al., 1976; Harwig et al., 1979; Huff et al., 1979; Zuber

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& LILLEHOJ, 1979; ABALAKA & ELEGBEDE, 1982). Prominent amongst these is *Aspergillus flavus* which produces the noxious and carcinogenic metabolites known as aflatoxins.

The use of gamma radiation alone for disinfestation of cereals and control of fungi infecting cereals and grains is well documented (KULIK & JUSTICE, 1966; POISSON & CAHAGNIER, 1974; KISS & FARKAS, 1977; ODAMTTEN, 1979, 1982) but it is desirable to undertake in vitro studies pertaining to the radiation response of the spores of *A. flavus* in combination with previous heating.

There are references in the pertinent literature on the novel approach for controlling fungal infection of stored products by the combination treatment of heat and irradiation. Radiation treatment combined with heat treatment (KISS & CLARKE, 1969; ROY et al., 1972; SOMMER et al., 1972; BRODRICK et al., 1976, 1977; LANGERAK & CANET-PRADES, 1979) was more effective than radiation treatment alone.

Recently, PADWAL-DESAI and co-workers (1976), showed that different strains of *A. flavus* (toxigenic and non-toxigenic) responded differently to heat treatment alone and to the combined treatment with gamma irradiation. This phenomenon had previously been reported by SOMMER and co-workers (1964). It is likely that the strain *A. flavus* NRRL 5906 infecting maize grains may show different response to heat treatment alone and to combined treatment with gamma irradiation. Such in vitro studies on this fungus using a model medium, maize meal agar (MMA), closely related to the product will provide the most relevant information necessary for the subsequent in vivo application studies on the product, maize grains. These experiments reported here were designed to provide pertinent information on the effect of mild heat (20–60 °C) alone and the combination treatment with gamma irradiation on the survival of spores of *A. flavus* NRRL 5906.

1. Materials and methods

1.1. Treatment of spores

1.1.1. Treatment in suspension (A). Spores of *A. flavus* Link NRRL 5906 were used in these studies. The method employed was essentially a modification of the procedure of LANGERAK and CANET-PRADES (1979). Spores of *A. flavus* were suspended in sterile Tween solution (0.2% Tween 80). The suspension was centrifuged at 4000 g for 15 min, the supernatant discarded with subsequent resuspending of the pelleted spores in fresh Tween. The centrifugation was repeated. The resultant suspension was then adjusted to a spore density of 10^6 to 10^7 spores per cm^3 with the help of a Hawksley B. S. 784 Haema cytometer (Hawksley and Sons Ltd., Sussex, England). Heating tubes

(∅ 2.5 cm, 16 cm long) containing 27 cm³ of sterile Tween solution used for each heating temperature (20, 40, 45, 48, 50, 52, 53, 55 and 60 °C) were maintained at the respective stated temperatures in water bath (P.M. Tamson N. V., Holland) and 3 cm³ aliquots of the stock suspension of spores were added. After heating for the pre-determined time intervals of 2.5, 5.0 and 10 min, the tubes were transferred to room temperature (cca 20 °C). Tubes which contained unheated spores (20 °C) were regarded as controls.

1.1.2. Treatment at different R. H. (B). The spores were harvested dry from a maize medium (200 g blended maize in 500 cm³ Erlenmeyer flask) and 0.1 g weight of spores transferred into sterile petri dishes (9.0 cm diameter). The spores were heated at 60 °C for 30 min in a forced heat micro-climatic chamber that enabled us to give the spores either a low humidity ($\leq$45% R. H.) or high humidity ($\geq$85% R. H.) during the heating period (ODAMTTEN et al., 1980). The lids of the petri dishes were eccentrically placed on the bottoms to allow maximum exposure of the spores to the prescribed treatment. The humidity and temperature during the heating period was monitored from a remote position (ODAMTTEN et al., 1980). After irradiation in the dry state, the spores were suspended in 30 cm³ of sterile Tween solution before plating on the agar medium. Other combined treated spores in petri dishes with nipples were kept at 80% R. H. for 4 and 8 days, respectively, before plating on Maize Meal Agar (MMA, 200 g maize powder in 1 dm³ distilled water) and Oxytetracycline Glucose Yeast Extract Agar Medium (OGYE, oxytetracycline 0.5 g; glucose 20 g; yeast extract 5 g; distilled water 1 dm³).

1.2. Radiation treatment

The irradiation was carried out not more than 30 min after heat treatment in the Pilot Plant for Food Irradiation at the International Facility for Food Irradiation Technology (IFFIT) Wageningen, The Netherlands. The dose rate was 2.6 kGy h^{-1}. The absorbed dose was checked by Fricke's dosimetry and clear perspex dosimeters. Test tubes containing 4.0 cm³ aliquots of heat-treated moist spores (Treatment A) were given 0.20, 0.50, 0.70 and 1.0, 1.5 kGy of gamma irradiation, resp., whilst the dry spores (Treatment B) were exposed to 3.5 and 4.0 kGy of gamma radiation.

1.3. Assessment of survivors in treated spores

Aliquots of 1.0 cm³ of the combination treated spores were diluted at the ratio 1 : 9 and were plated out on MMA and OGYE media employing the conventional microbiological technique of serial dilution. After an incubation period of 3 days at 28 °C, the log survival was plotted on linear graph paper against radiation dose. The D_{10} values and correlation coefficients of the combination treated samples were calculated where appropriate.

2. Results

2.1. Effect of heating and in combination with radiation on spores in Treatment A

The effects of the various temperatures and heating times in Treatment A are illustrated in Fig. 1. There was no difference in the surviving population in the range of 20–45 °C after 2.5, 5.0 and 10 min heating periods. Subsequent increase in heating temperature from 45 °C to 49 °C for 2.5 and 5.0 min did not reduce the surviving population of spores. The adverse effect of heating on the spores generally started after the spores had been treated at about 52 °C for at least 2.5 min. At temperatures higher than 50 °C, a reduction in the surviving population was recorded when the heating time was increased from 2.5 to 10 min. The spores were, however, completely killed after heating them for 5 min and 10 min at 59 °C and 58 °C, respectively. Thus heating alone

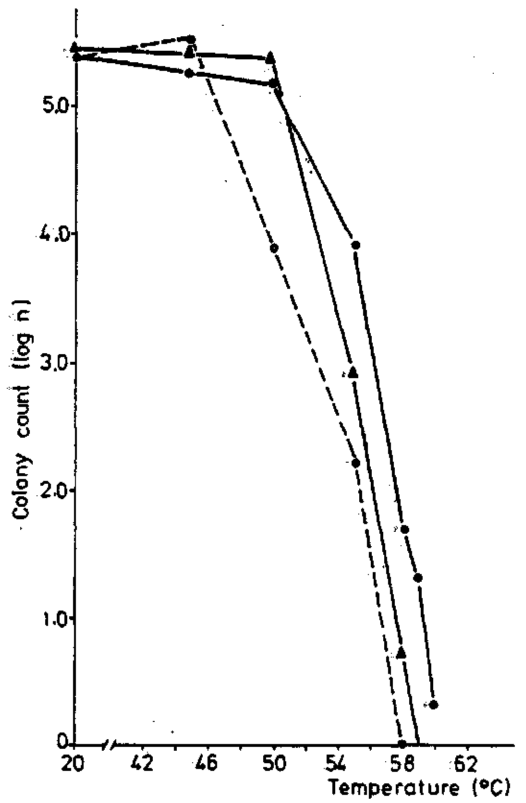

Fig. 1. Graph showing effect of heating temperature and duration of heating on the survival of spores of *A. flavus* in suspension. (Medium used was MMA). –●–: heating for 2.5 min; –▲–: heating for 5 min; – – ○ – –: heating for 10 min

for at least 5 min at 59 °C is considered sufficient to inactivate the spores of *A. flavus* treated in suspension.

When irradiation was (0.25–1.5 kGy) combined with heating, a synergistic effect was obtained even at the sub-lethal temperatures from 50 °C to 53 °C where heating alone had a slight effect on the spores (Figs. 2–4). Generally 5 min heating at 53 °C in combination with 0.75 kGy of gamma radiation resulted in complete inhibition of spore germination (Fig. 2).

The D_{10} values obtained are presented in Table 1. The correlation coefficients were in the range of 0.930 to 0.999.

There was, generally, a decrease in D_{10} values when the sub-lethal heating temperature of 50 °C was exceeded and similar D_{10} values of 0.19 and 0.18 kGy were obtained after combination treatment with 0.75 kGy for 5 and 10 min heating times, resp., at 53 °C.

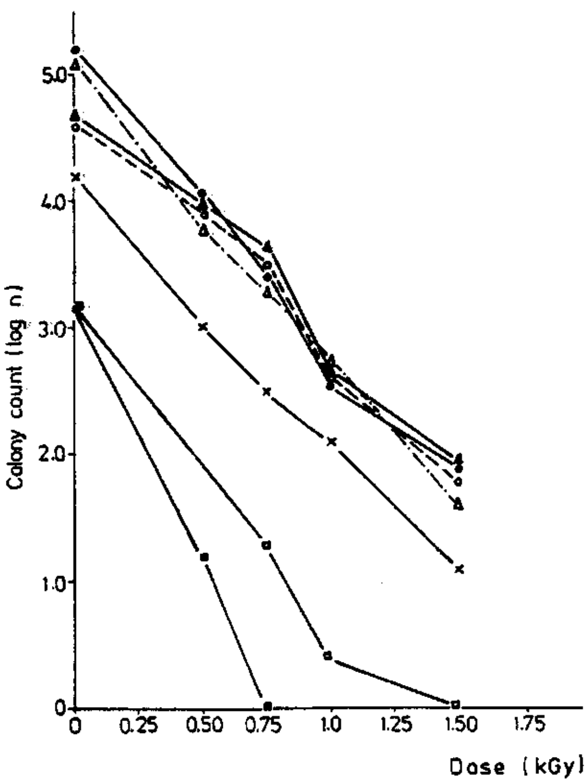

Fig. 2. Effect of 2.5 min heating of spores of *A. flavus* in suspension at the indicated temperatures, in combination with gamma radiation doses (0.50–1.50 kGy) on the survival of spores. (Incubation medium was MMA.) –●–: 20 °C; --○--: 40 °C; –▲–: 45 °C; —·△·—: 48 °C; –×–: 50 °C; –□–: 52 °C; –■–: 55 °C

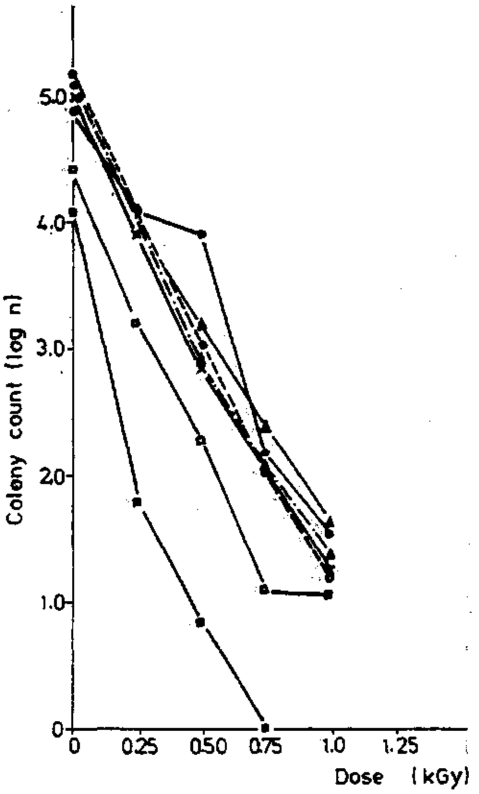

Fig. 3. Effect of 5 min heating of spores of *A. flavus* in suspension at the indicated temperatures, in combination with gamma radiation doses (0.25–1.0 kGy) on the survival of spores. (Incubation medium was MMA.) —●—: 20 °C; --○--: 40 °C; —▲—: 45 °C; —△—: 48 °C; —×—: 50 °C; —□—: 52 °C; —■—: 53 °C

Table 1

Comparative D_{10} *values (kGy) of combination treatment of heating (different temperatures and times) and gamma radiation, resp.*
(Spores of *Aspergillus flavus*)

Heating temperature (°C)	D_{10} values at indicated heating time periods (kGy)		
	2.5 min[a]	5.0 min	[illegible] min
20	0.43	0.29	0.25
40	0.52	0.28	0.28
45	0.51	0.27	0.29
48	0.44	0.27	0.25
50	0.50	0.29	0.25
52	0.42	0.29	0.19
53	—	0.19	0.18
55	0.32	—	—

[a] Data for 2.5 min were obtained from a separate experiment

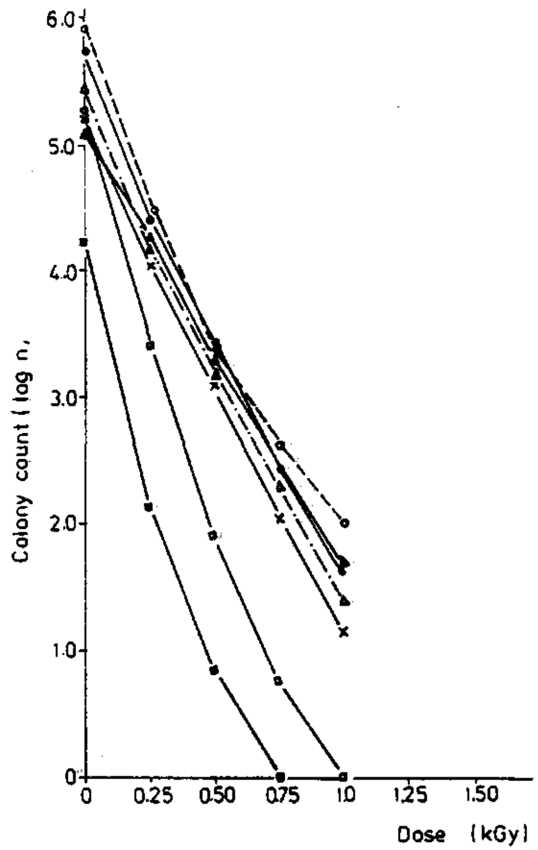

Fig. 4. Effect of 10 min heating of spores of *A. flavus* in suspension at the indicated temperatures, in combination with gamma radiation doses (0.25–1.0 kGy) on the survival of spores. (Incubation medium was MMA.) —●—: 20 °C; --○--: 40 °C; —▲—: 45 °C; —△—: 48 °C; —×—: 50 °C; —□—: 52 °C; —■—: 53 °C

2.2. Effect of heating only and in combination with radiation on dry spores in Treatment B

The effect of heating alone (60 °C for 30 min) and the combination treatment with radiation (0, 3.5 and 4.0 kGy) on the dry spores is illustrated in Figs. 5 and 6. The efficacy of heat in inactivating spore germination depended strongly on the ambient humidity during heat treatment. Heating alone at 60 °C for 30 min at high humidity (≥85% R. H.) reduced the mould count on OGYE (Fig. 5) and MMA (Fig. 6) by 3 log cycles whilst a parallel treatment at low humidity (≤45% R. H.) conditions did not appreciably reduce the

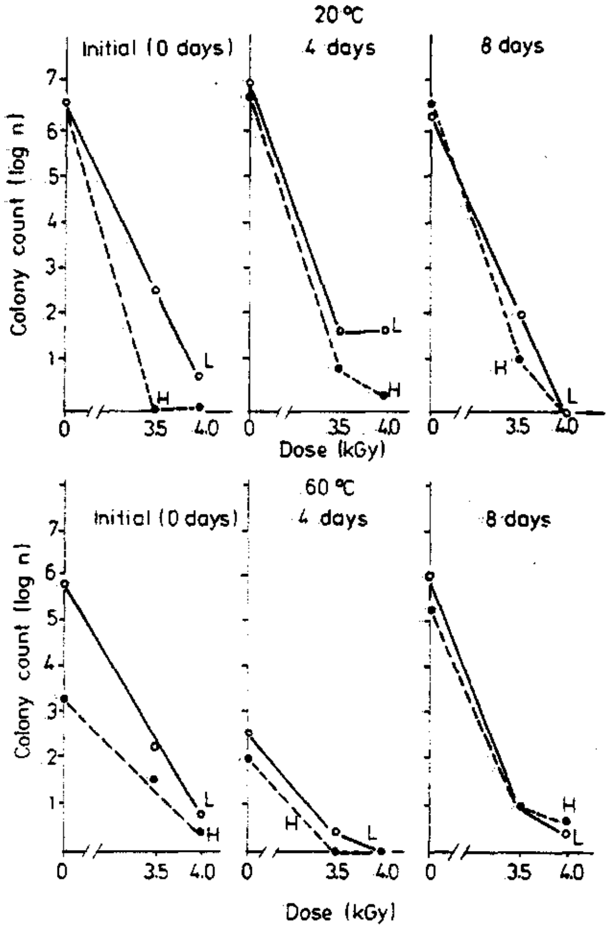

Fig. 5. Effect of irradiation of the survival of unheated (control) spores of *A. flavus* (top) kept after treatment at 80% R.H. ambient humidity for 0, 4, and 8 days, respectively, before plating on OGYE medium; and (bottom) the effect of the combination of heat (60 °C for 30 min) with irradiation on the survival of spores of *A. flavus* stored at 80% R.H. for 0, 4 and 8 days, respectively, before plating on OGYE. L: humidity ≤45% R.H.; H: humidity ≥85% R.H.

spore population. However, when the combination treated spores were stored at 80% R. H. for 4 days before plating, spore germination was considerably reduced (by 3 log cycles) even under low-humidity heating conditions. The lethality of the combined treatment was evident after 4 days but thereafter, the spores rather curiously recovered after 8 days storage at 80% R. H. Again, similar results were obtained on the two solid media, namely OGYE and MMA.

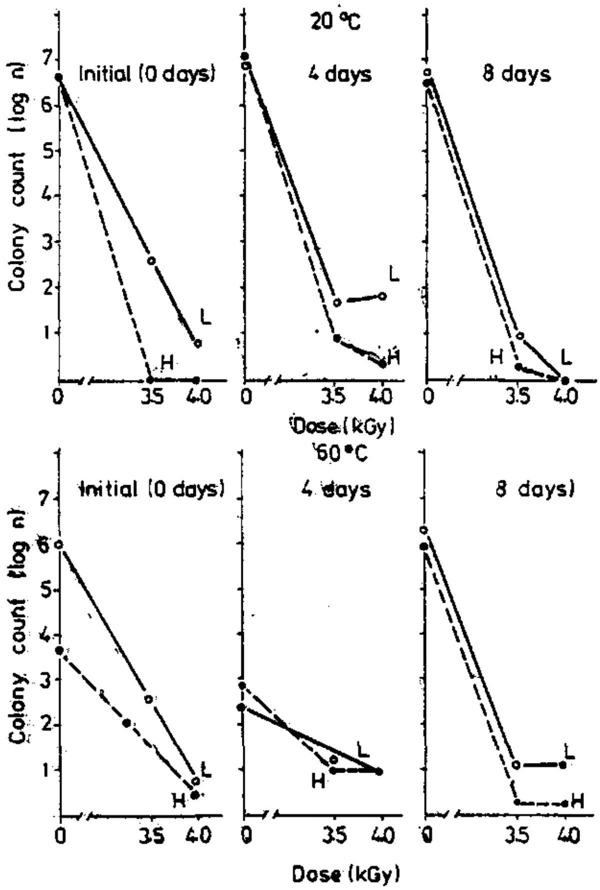

Fig. 6. Effect of irradiation on the survival of unheated (control) spores of *A. flavus* (top) kept, after treatment, at 80% R.H. ambient humidity for 0, 4 and 8 days, respectively, before plating on MMA medium; and (bottom) the effect of the combination of heat (60 °C for 30 min) with irradiation on the survival of spores of *A. flavus* stored at 80% R.H. for 0, 4 and 8 days respectively before plating on MMA medium. L: humidity ≤45% R.H.; H: humidity ≥85% R.H.

The unheated (20 °C) spore population remained the same with a slight increase on the 4th day at 80% R. H. There was no distinct difference between unheated and unirradiated spores treated under low and high humidity conditions, respectively. In all instances, the size of colonies was larger on OGYE than on MMA.

Radiation treatment was more effective after exposing the spores to hot air of high humidity (≥85% R. H.) before irradiation than when low humidity (≤45% R. H.) was applied (Figs. 5 and 6).

3. Conclusions

The report of MOYHUDDIN and SKOROPAD (1975) stated that the synergistic effect of heat and gamma radiation on the conidia of *A. flavus* Link var. *columnaris* Rapper and Fennel increased with an increase in temperature (35–55 °C applied for 20 min) and radiation dose (0.25–1.25 kGy) and that this increase was more pronounced at temperatures above 45 °C. They found that heating the conidia at 50 °C for 20 min followed by irradiation was most effective. At 1.0 and 1.25 kGy gamma radiation, there were no survivors. The conidia of *A. flavus* Link (toxigenic) were more resistant to heat treatment at 50 °C for 5 min than conidia of *A. flavus*, *A. oryzae* (non toxigenic). However, survival of both strains was not affected by being exposed to 50 °C for 75 min (PADWAL-DESAI et al., 1976). Heat treatment at 55 °C for 5 min reduced the conidial population to 6.5% and 0.12% in the toxigenic and non-toxigenic strains, respectively.

Our results showed that the adverse effect of heating on the spores (conidia) of *A. flavus* NRRL 5906 began at a temperature greater than 52 °C when the heat was applied for 2.5 and 5 min, resp., (Fig. 1) and that heating at 59 °C for at least 5 min is considered sufficient to inactivate spore germination. Generally, 5 min heating at 53 °C in combination with 0.75 kGy of gamma radiation resulted in complete inactivation of spores of *A. flavus* (Fig. 3). The fact that different strains of a species of fungus may vary in sensitivity to heat and irradiation combination treatment has been shown by other workers (SOMMER et al., 1964; MOYHUDDIN & SKOROPAD, 1975; WEBB et al., 1969).

From the economic point of view, lowering the radiation dose by the combination process is advantageous and desirable for the effective control of germinating spores of fungi. This type of in vitro studies will be relevant for products like fruits and vegetables which can be immersed directly in moderately warm water without any adverse effect on the plant tissue. It would, in this instance, be superfluous to immerse the product for more than 5 min in the warm water since no significant difference was found between the D_{10} after 5 and 10 min heating at 53 °C (Table 1).

Our experimental results show that spores of *A. flavus* NRRL 5906 irradiated in suspension (Treatment A) were more radiation sensitive than those treated at different humidity conditions (Treatment B). Whilst a sublethal temperature of 53 °C applied for 5 min in combination with 0.75 kGy inactivated spores suspended in Tween solution, a dose of 4.0 kGy was required in combination with prior heating under high humidity conditions ($\geq$85% R. H.) at 60 °C for 30 min to achieve the same purpose. These findings are in agreement with the report of NYERGES-ROGRÜN (1975) whose test fungus was *Penicillium purpurogenum* Stoll; Strain No. 787.

We recorded a higher killing effect when the spores were heat-treated under high humidity ($\leq$85% R. H.) conditions before irradiation than when low humidity ($\leq$45% R. H.) prevailed during the heating prior to irradiation. Thermal inactivation of microorganisms is due to the denaturation of enzymes and other proteins. Perhaps the stress of heat on essential biological molecules of the spore is augmented by high humidity conditions. There is intensified diffusion of heat and moisture as well as increased target size due to thermal expansion of cell wall. It is well documented that in addition to the temperature and duration of treatment, humidity strikingly affects lethality of heat applied. Moist heat shows greater lethality for two reasons; i.e. enzymes are more readily coagulated and hydrated, and heat is transferred more readily in humid air (HAWKER et al., 1952). This sensitization renders the spores more vulnerable to irradiation affecting the nucleus of the spores. The damage to cellular DNA is one of the major factors responsible for radiation lethality. We cannot explain from our present data, the recovery of heated (60 °C for 30 min) and unirradiated spores after being at 80% R. H. for 8 days.

Studies of this nature, using moist heat (high humidity) to inactivate fungi on stored products in combination with gamma radiation will be more relevant to cereal grains and grain products which cannot be dipped into water and therefore furnish important information for direct application. The dose and heat requirements may however depend on the type of fungi and product and needs to be determined in a preliminary experiment.

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4. Preliminary studies of the effects of heat and gamma irradiation on the production of aflatoxin B_1 in static liquid culture, by *Aspergillus flavus* link NRRL 5906

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Aflatoxin B_1 production by a strain of *Aspergillus flavus* NRRL 5906 was examined in static liquid culture in maize meal broth (MMB) and maize meal broth supplemented with 2% glucose and 2% peptone (AMMB). Erlenmeyer flasks were inoculated with 1.0 ml aliquots of fungal spores which had been heat-treated (60°C for 30 min) under low humidity (< 45% R.H. dry heat) or high humidity conditions (> 85% R.H., moist heat) followed by gamma irradiation with either 0.0, 3.5 or 4.0 kGy. AMMB supported 6–17 times more vegetative growth (depending on the heat and dose combination) than spores incubated in MMB alone. High inoculum size of control unheated spores (log CFU/g, 6.9) yielded the least aflatoxin B_1 in flasks containing AMMB (8.2–19.3 μg/ml). A dose of 3.5 kGy reduced by 3.2–3.8 log cycles the viable inoculum of control unheated spores, resulting in 2–5 fold increase in aflatoxin B_1 formed in flasks containing AMMB. Increasing the applied load to 4.0 kGy, however, reduced aflatoxin B_1 levels formed in AMMB to similar or lower levels than found in flasks inoculated with control unirradiated spores. Combination treatment of *A. flavus* with dry heat and 3.5 kGy reduced the spore inoculum size by about 4 log cycles and yielded the highest amount (41.1 μg/ml) of aflatoxin B_1 in AMMB. However, moist heat treatment of spores receiving the same dose (3.5 kGy) reduced toxin level formed by 25%. Aflatoxin B_1 formation by *A. flavus* spores incubated in AMMB was completely prevented by a combination treatment of moist heat and 4.0 kGy of gamma irradiation. This same treatment attenuated aflatoxins B_2, G_1 and G_2 production which are formed with B_1 by *A. flavus* NRRL 5906. Spores raised in all flasks containing MMB did not form aflatoxin except when the medium MMB was autoclaved twice at 121°C for 15 min.

Key words: Heat, moist and dry; Gamma irradiation; Aflatoxin B_1; *Aspergillus flavus* NRRL 5906

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Introduction

Aflatoxins are secondary metabolites of the mould *Aspergillus flavus* and *Aspergillus parasiticus* that produce a toxic reaction in humans and other mammals (Tulpule et al., 1964; Wogan, 1973).

Experimentally, aflatoxins have been produced on numerous food products including cereals, grains, whole wheat and rye bread, cheese, meat, groundnut and nut products and fruit juice (Frank, 1968; Detroy et al., 1971; Zuber and Lillehoj, 1979), copra and cotton seed (Christensen and Schneider, 1950; Ashworth et al., 1971; Simpson et al. 1973).

A practical approach to protecting food against aflatoxin contamination is by preventing the fungi from forming mycotoxin. Gamma irradiation of food for preservation has been proposed and studied for the past twenty-five years as an alternative means of food preservation. This has led to a series of investigations on the safety of food in relation to aflatoxin production by residual mycoflora after gamma irradiation treatment.

Studies by Jemmali and Guilbot (1970), Schindler et al. (1980) and Pnyadarshini and Tulpule (1976) indicated an increase in aflatoxin production after gamma irradiation. Several closely related papers by Applegate and Chipley (1974a, b) also presented evidence of increased aflatoxin production by *A. flavus* after gamma irradiation.

A novel approach to control of fungal contamination of stored products is by combination treatment of heat and irradiation. This enables one to use a much lower dose in combination with mild heat treatment to achieve very high level inactivation of fungal spores. Radiation treatment combined with heat has been found to be more effective than radiation treatment alone (e.g. Roy et al., 1972; Langerak and Cañet-Prades, 1979; Odamtten et al., 1985, etc.).

Our objective in these investigations was to provide data on the effect of the combination treatment of heat (60°C for 30 min) applied under low ambient humidity (< 45% R.H.) or moist heat applied under high ambient humidity (> 85% R.H.) and gamma irradiation (0.0, 3.5 and 4.0 kGy) on production of aflatoxin B_1 in submerged static culture medium prepared from blended maize grains.

Materials and Methods

Treatment of dry spores

Fungal spores of *A. flavus* Link NRRL 5906 were harvested dry from a previously sterilized (121°C for 15 min) Dutch variety yellow maize grains (200 g blended maize in 500 ml Erlenmeyer flasks). The seven days old spores incubated at 28°C were separated from the maize medium by sieving with a plastic seive and 0.1 g spores transferred into sterils Petric dishes (90 mm diameter).

The spores were heated at 60°C for 30 min. in a chamber that enabled us expose the spores to either dry (< 45% R.H.) or moist (> 85% R.H.) heating conditions

(Odamtten et al., 1980). Fungal spores that were maintained at 20°C during the prescribed humidity treatments served as controls. The lids of the Petri dishes were eccentrically placed on the bottom to allow maximum exposure of the spores to the prescribed treatment. After irradiation (see below) in the dry state, the spores were washed with 30 ml of sterile Tween solution (2 g Tween-80, 1 l, distilled water) to get the spores into suspension. Aliquots of 1 ml from the stock suspension of each treatment combination were used in inoculating 250 ml Erlenmeyer flasks. Each flask contained 25 ml of either maize meal broth MMB (200 g maize; blended, strained and supernatant made up to 1 l with distilled water) or ammended maize meal broth AMMB (200 g maize, blended, strained and supernatant made up to 1 l with distilled water and added 20 g peptone and 20 g glucose). There were five replicates for each treatment combination and the flasks were incubated in darkness at 28°C for 4 days.

Radiation treatment

Irradiation, as a rule, was carried out not more than 30 min. after the heat treatment (in order not to lose the synergistic effect of the combination treatment) in the Pilot Plant for Food Irradiation at the International Facility for Food Irradiation Technology (IFFIT) Wageningen, the Netherlands. The dose rate was 5.5 krad min^{-1} and the absorbed dose was checked by clear Perspex Dosimetry. We applied doses of 0, 3.5 and 4.0 kGy of gamma radiation to the spores.

Assessment of initial survivors in treated spores

Aliquots of 1.0 ml of the stock suspension of the treated spores were diluted up to $1:10^5$ and plated out on maize meal agar, MMA (200 g maize grains, blended, strained and made up to 1 litre with distilled water, 20 g agar was added) and ammended maize meal agar, AMMA (200 g maize grains, blended strained and made up to 1 l with distilled water, 20 agar, 20 g glucose, 20 g peptone) using the serial decimal dilution technique. After incubation at 28°C for 3 days, the log survival of the treated spores was determined.

Extraction of aflatoxin from the culture filtrates

A modification of the method of Ko, (1974) was followed. With a volumetric pipette, 50 ml chloroform was added to each of the flasks containing the culture filtrate, from which mycelium had been harvested. The extraction of aflatoxin into the chloroform layer was promoted by shaking (100 rev min^{-1}) for 30 min. In a Gallenkamp model orbital shaker. The chloroform extract phase was simply separated from the rest of the medium by mean of a separating funnel. After this, the chloroform extract was evaporated to dryness in a Rotavapor vacuum rotation evaporator (Buchi, Switzerland). The residue was redissolved in 5.0 ml of chloroform and the contents of the five replicate flasks were pooled and kept in 250 ml Erlenmeyer flasks covered with cotton wool and aluminium foil at 2°C for one night or more because determination of aflatoxin content of the filtrate by thin layer chromatography could not be completed on the same day.

Quantitative estimation of aflatoxin B_1 in culture filtrates

Samples of the extracted aflatoxin from the culture filtrate of treated spores were spotted on MN-KIESEGEL G-HR thin layer chromatography (Machery, Nage and Company, Duran, F.R.G.) using a Desaga Microdoser (Heidelberg, F.R.G.) with acetone : chloroform (1 : 9) and developing solvent. The intensity of the fluorescing spots was measured with a Vitraton TLD-100 densito-meter (Vitraton Scientific Instruments, Dieren, The Netherlands) equipped with an integrator recorder. The Amount of aflatoxin B_1 was calculated by comparison of the integration units between the peak area of spots of unknown samples and those of known standard spots on the same plate.

Assessment of dry weight of mycelium and pH of cultures

After incubating the cultures for 4 days at 28°C in darkness, growth in the liquid medium was assessed by estimating the dry weight of the harvested mycelium. Mycelium collected on a previously weighed and dried filter paper was dried in an oven at 75°C for 24 h (until constant weight was attained). The filters paper carrying the dried mycelium were then re-weighed after cooling in a desiccator. The dry weight of the mycelium was estimated by difference. The pH of each of the culture filtration was determined by a Marius pH Meter Type 52A.

Results

Effect of the combination treatment on vegetative growth and sporulation

Vegetative growth of *A. flavus* in flask containing AMMB (Fig. 2) was about 6–17 times greater than in MMB (Fig. 1), depending on the heat and dose combination (Figs. 1 and 2). In both types of media the lowest dry weight of mycelium was recorded in flasks containing spores treated with a combination of moist heat (> 85% R.H.) and 4.0 kGy.

Generally, the final pH of culture filtrates of *A. flavus* incubated in MMB was lower than the initial pH of 5.5, with the attendant restriction of both growth and toxin formation by the fungus. In flasks containing AMMB however, the final pH of the filtrate shifted from initial pH 5.3–5.5 to become less acid (6.3–6.8) in flasks containing control spores (unheated 20°C, and heat-treated 60°C under both low, < 45% R.H. and high, > 85% R.H. humidity conditions). Culture filtrates from control spores exposed to 4.0 kGy became more acidic (pH 3.9–4.1) except with moist heat-treated spores, where there was only a slight shift from the initial pH of 5.3–5.6. The lowest mycelial dry weight was recorded in the latter flasks.

The spore inoculum used in inoculating the flasks containing either MMB or AMMB was largest in the controls. These inoculum sizes shown in Table I were

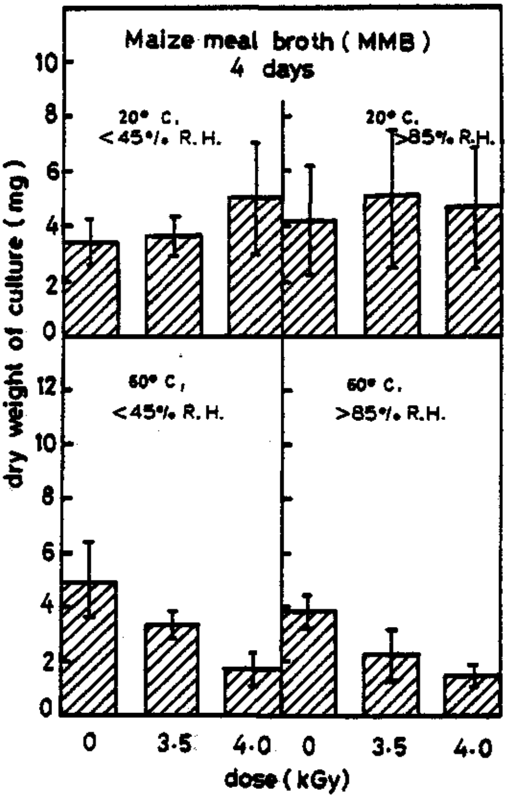

Fig. 1. Comparative mycelial dry weight yield of cultures of *A. flavus* incubated in maize meal broth (MMB) for 4 days at 28°C.

subsequently reduced by 3.3–5.8 log cycles by 4.0 kGy of gamma irradiation. Moist or dry heating in combination with 4.0 kGy also reduced the inoculum size by 3.4 and 5.6 log cycles respectively (Table I). In flasks containing large inocula (unheated and unirradiated controls), vegetative growth predominated over sporulation and cultures were whitish in colour as compared to the abundant green to dark green spores formed in flasks inoculated with combined-treated spores.

Aflatoxin formation

As seen from Table I, no aflatoxin was formed in MMB irrespective of the treatment given to the spores. Sporulation was generally poor in these flasks. The toxin was however formed freely into the medium when MMB was sterilised twice at 121°C for 15 min (results not included).

Table I shows that maize meal broth supplemented with 2% glucose and 2% peptone (AMMB) supported the formation of low (8.2 $\mu g/ml$) aflatoxin B_1 in flasks

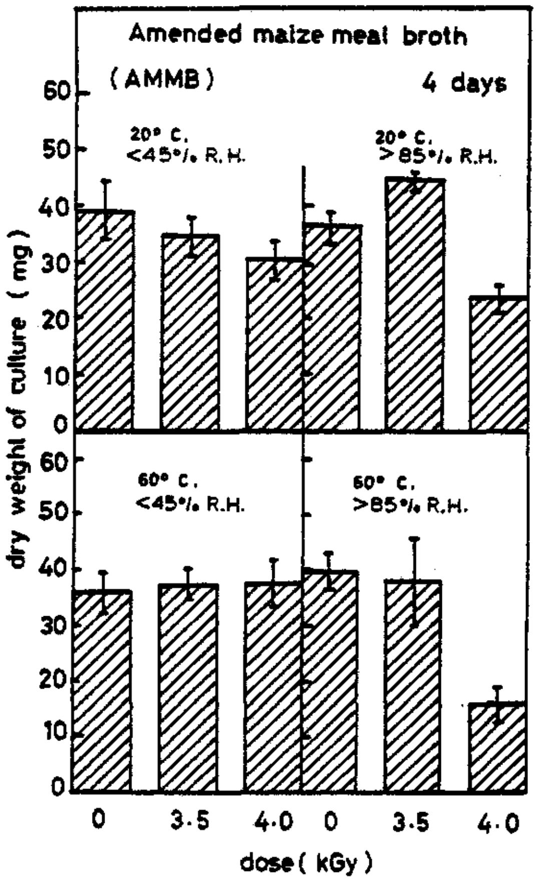

Fig. 2. Comparative mycelial dry weight yield of cultures of *A. flavus* incubated in amended maize meal broth (AMMB) for 4 days at 28°C.

inoculated with unirradiated *A. flavus* spores heat treated under dry humidity conditions (< 45% R.H.). Control (20°C, unirradiated) spores heat-treated under high humidity (> 85%R.H.) for 30 min formed 19.3 μg/ml aflatoxin B_1 into AMMB. Dry and moist heat-treated spores under low (< 45% R.H.) and high (> 85% R.H.) ambient humidity conditions formed higher levels of aflatoxin B_1 (28.8 μg/ml and 40.0 μg/ml respectively) than the unheated spores and the resulting cultures were green to white in some flasks. Dark green sores banded the periphery of the mycelial mat. Irradiation alone with 3.5 kGy increased by 4–5 3-fold the aflatoxin B_1 formation by the control unheated spores treated under low (< 45% R.H.) humidity conditions. The parallel treatment under high humidity conditions (> 85% R.H.) doubled the toxin level. Dry heat-treated spores in combination with 3.5 kGy formed aflatoxin B_1 about one and a half times that formed by heating alone. The only exception was in flasks containing moist

TABLE I

Relationship between inoculum size, aflatoxin B_1 formation, sporulation and colour of control and combined treated *A. flavus* spores incubated in indicated medium for 4 days at 28°C

Type of treatment (temperature (°C) and humidity (% R.H.))	Dose (kGy)	Inoculum size (log_{10} C.F.U./g)		Aflatoxin B_1 (µg/ml)		Colour of culture		Sporulation [c]	
		MMB	AMMB	MMB [a]	AMMB	MMB	AMMB	MMB	AMMB
20°C (< 45%)	0.0	6.2	6.9	ND	8.2	White	White/Yellow	–	–
	3.5	3.0	3.1	ND	38.5	Dark green	Dark green	+	+ + +
	4.0	2.9	1.7	ND	11.2	Dark green	Dark green	+	+ + + +
20°C (> 85%)	0.0	5.9	6.9	ND	19.3	White	White	–	–
	3.5	1.7	2.6	ND	39.0	Dark green	Dark green	+	+ + +
	4.0	0.1	1.7	ND	6.5	Dark green	Dark green	+	+ + + +
60°C (< 45%)	0.0	6.7	6.1	ND	28.8	Green	Green	+ +	+ + +
	3.5	3.4	2.9	ND	41.5	White/Yellow	Dark green	+ + +	+ +
	4.0	2.0	2.7	ND	32.5	Dark green	Dark green	+ + +	+ +
60°C (> 85%)	0.0	7.1	5.8	ND	40.0	Green	Green	+	+ + +
	3.5	2.7	1.8	ND	30.0	Dark green	Dark green	+	+ +
	4.0	2.0	0.2	ND	0.0	* [b]	* [b]	–	–

[a] Single sterilization
[b] No sporulation
[c] Sporulation: – nil, + sparse, + + moderately abundant, + + + abundant, + + + + very abundant, ND not detected.

heat-treated spores (60°C for 30 min at > 85% R.H.) where toxin level was reduced by 25%. Increasing the applied dose to 4.0 kGy in all cases decreased level of toxin formed to concentrations nearly the same or lower than that obtained in the unirradiated samples (Table I). The best treatment combination to prevent aflatoxin formation was moist heat (> 85% R.H.) at 60°C for 30 min in combination with 4.0 kGy (Table I). Wherever aflatoxin B_1 was formed, B_2, G_1 and G_2 were formed as well. The treatment that prevented aflatoxin B_1 formation (moist heat, 60°C for 30 min, > 85% R.H.) in combination with 4.0 kGy attenuated the formation of aflatoxins B_2, G_1 and G_2 as well.

Discussion

The abundant vegetative growth of *A. flavus* NRRL 5906 on AMMB as compared to MMB could be attributed to the addition of 2% glucose and 2% peptone to the basal medium. Glucose appears not only to serve as the carbon source for aflatoxin synthesis but also to play a role in regulation the induction of the enzymic pathway responsible for the biosynthesis of the mycotoxin (Abdoullahi and Buchanan, 1981). Mateles and Adyes (1965) demonstrated that a correlation exists between increasing concentration of glucose from 1–4% and the increase in amount of aflatoxin B_1 formed from 25 mg/l to 58 mg/l. Peptone is an excellent source of amino acids that can be catabolised to pyruvate or acetyl-coA for use by the fungus.

However, good vegetative growth by *A. flavus* is not always with abundant aflatoxin formation. The heavy inoculum used in the controls resulted in vegetative growth predominating over sporulation i.e. the cultures were within, very low aflatoxin B_1 was formed and the culture filtrate was clear to slightly reddish. Culture filtrates of MMB, where aflatoxin B_1 was not produced were also clear and sporulation was sparse to nil. Detroy et al. (1971) showed that aflatoxin synthesis occurs during the period of intense sporulation of the fungus and that good aflatoxin production correlates with yellowing of mycelium and medium. In flasks which failed to yield aflatoxin, the mycelium remained white and the medium was either clear or slightly reddish (Mateles and Adye, 1965). This is confirmed by our findings.

Reduction in inoculum size by 3.0–4.0 log cycles after gamma irradiation (3.5 kGy) followed was by abundant sporulation and enhanced aflatoxin B_1 formation in flasks containing AMMB after 4 days of incubation, indicating that the formation of aflatoxin B_1 is inversely related to the spore concentration.

Aflatoxin B_1 formation was completely inhibited by a combination treatment of moist heat (60°C for 30 min) applied under high ambient humidity (> 85% R.H.) conditions and 4.0 kGy of gamma irradiation. This treatment could also prevent production of aflatoxins B_2, G_1 and G_2 by *A. flavus* NRRL 5906. Recent findings by Odamtten et al. (1985) confirm that in addition to the temperature and duration of treatment, humidity strikingly affects the lethality of heat applied.

Flasks containing spores incubated in MMB failed to form the toxin irrespective of the type of treatment the spores received. However, after double sterilization of the medium MMB, aflatoxin B_1 was formed in detectable quantities in all cultures but that spores treated with moist heat (60 °C for 30 min. under humid > 85% R.H. conditions) in combination with 4.0 kGy. Lillehoj et al. (1974) observed the production of aflatoxin by *A. flavus* on various autoclaved corn media, whole corn being an excellent substrate, whilst Detroy et al. (1971) demonstrated maximum yield of aflatoxin B_1 (700–900 mg kg^{-1}) on autoclaved wheat, rice, cotton and corn. Several environmental, nutritional and genetic factors considerably influence the type and amount of aflatoxins produced by moulds of the genus *Aspergillus* (Maggon et al., 1977; Zuber and Lillehoj, 1979). For *A. flavus* and *A. parasiticus*, the optimal nutritional availability of certain trace metals in a crucial factor (Maggon et al. 1977). The stimulatory effect of zinc on aflatoxin production is well documented (Lee et al., 1966; Maggon et al., 1977; Lillehoj et al., 1974; Gupta and Venkitasubramanian, 1975; Obidoa and Ndubuisi, 1981).

Trace elements in maize occur predominantly in the germ fraction (Garcia et al., 1972). Phytic acid in the germ strongly binds other elements in addition to zinc (O'dell and Savage, 1960). The bound elements are not available biologically. The failure of *A. flavus* NRRL 5906 spores to form aflatoxin B_1 on MMA could probably be attributed to the binding action of phytic acid in maize with zinc. Although we did not estimate the amount of Zn^+ present in the autoclaved MMB, we surmise that on autoclaving of MMB, zinc is released but is not enough to permit the synthesis of detectable amounts of aflatoxin B_1. However by autoclaving twice, phytic acid is presumably destroyed by the excessive heat releasing sufficient zinc for aflatoxin synthesis. In future studies, other local maize varieties will be used as cultural media to compare them with the results reported in the present paper.

We have shown here that combination treatment with moist hot air (60 °C for 30 min) administered at high humidity (> 85% R.H.) in combination with 4.0 kGy prevented not only aflatoxin B_1 production but also attenuated

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5. INFLUENCE OF INOCULUM SIZE OF *ASPERGILLUS FLAVUS* LINK ON THE PRODUCTION OF AFLATOXIN B_1 IN MAIZE MEDIUM BEFORE AND AFTER EXPOSURE TO COMBINATION TREATMENT OF HEAT AND GAMMA RADIATION

G.T. Odamtten, V. Appiah, and D.I. Langerak.

Abstract

This paper examined the influence of inoculum size of *Aspergillus flavus* Link NRRL 5906 on growth and production of aflatoxin B_1 in static culture, double-autoclaved maize meal broth (MMB) and maize meal broth ammended with 2% peptone and 2% glucose (AMMB). Dry spores of the fungus were heat-treated in a heat-treatment chamber that enabled us to apply precisely 60 °C for 30 min. at an ambient humidity of either <45% R.H. (dry heat) or >85% R.H. (moist heat) during the heating period. Controls were maintained at 20 °C during the humidity treatment and samples were irradiated within 30 min. after the treatment, with 0.0, 3.5 or 4.0 KGy of gamma irradiation.

Vegetative growth of untreated controls in culture was independent of inoculum size with which flasks were inoculated and reduction in inoculum size by serial dilution (3-4 log cycles) resulted in 3-12 fold increase in aflatoxin production. Moist heat reduced vegetative growth by two-thirds and subsequent 3-4 log cycles reduction of inoculum size impaired toxin-formation potential of most surviving fraction of heat treated spores. Irradiation with 3.5 KGY reduced inoculum size by 3-5 log cycles and resulted in enhanced aflatoxin B_1 production but this effect was similar to what existed with serial dilution of control spores. Irradiation and its combination with heat may not have any direct effect on aflatoxin B_1 production but on the size of inoculum in relation to viable spores. Maximum accumulation of aflatoxin B_1 was generally in 4 days in AMMB and 8 days in MMB. A combination of moist heat (60 °C for 30 min. under >85% R.H.), 4.0 KGy of gamma irradiation attenuated aflatoxins production in flasks containing both MMB and AMMB.

Key words: Inoculum size; *Aspergillus flavus* Link NRRL 5906; vegetative growth and aflatoxin B_1 formation; maize meal broth; combination treatment of heat and gamma irradiation.

5.1 Introduction

Among the field and storage mycoflora associated with cereal grain products, members of the genus *Aspergillus* are the most predominant followed by *Penicillium* (Christensen and Kaufmann, 1969; Moubasher et al., 1972; Odamtten, 1981). Two economically important aflatoxin producing strains *Aspergillus flavus* and *A. parasiticus* are ubiquitous associated with other wide variety of stored commodities (Diener and Davies, 1977).

Over the past two decades, considerable importance has been attached to the presence of aflatoxins in food and feeds because of their carcinogenic, mutagenic and teratogenic nature (Goldblatt, 1969; Tawes et al., 1972; Enomoto and Saito, 1972; Austwick, 1975; Heathcoate and Hibbert, 1978). The factors influencing aflatoxin production have been widely evaluated in both natural and synthetic substrates (Sharma et al., 1980). Aflatoxin production is known to depend upon the strain of the organism and factors such as composition of the medium (Mateles and Adyel, 1965; Davis et al., 1966; Schroeder, 1966; Detroy et al., 1971; Abdoullahi and Buchanan, 1981), temperature (Schroeder and Hein, 1967, 1968; Eldridge, 1968; Stultz and Krumperman, 1976), moisture level (Lopez and Christensen, 1967; Trenk and Hartman, 1970; Diener and Davis, 1977; Behere et al., 1978), oxygen tension and time of incubation (Landers et al., 1967; Maggon et al., 1977).

Synthetic media have routinely supported minimal aflatoxin production (1-60 mg of B_1 per kg of medium) whereas maximum yields (700-900 mg of B_1 per kg) occur on such commodities as autoclaved wheat, rice, cotton seed and corn (Detroy et al., 1971). Hesseltine and co-workers (1966) in their review of aflatoxin formation by *A. flavus* regarded maize as a satisfactory substrate for aflatoxin production. Interest in control of microbial spoilage of food by gamma radiation, led to a series of studies of the effect of irradiation on aflatoxin production potential of *A. flavus* in irradiated foods (Jemmali and Guilbot, 1969b; 1970a,b; Applegate and Chipley, 1974a,b; Schindler et al., 1968) etc. Some of these workers (e.g. Jemmali and Guilbot, 1969b, 1970a,b) reported of an increase in aflatoxin production after gamma irradiation of *A. flavus* spores, whilst report of Applegate and Chipley (1973) stated that irradiation did not induce production of aflatoxin on either wheat or synthetic medium. Sharma et al. (1980) showed that for *A. parasiticus* NRRL 3145, the reduction in the number of spores by 4-5 log cycles either by serial dilution or by gamma irradiation caused a two-fold increase in the aflatoxin production. It was apparent that the irradiation effect was similar to that obtained by dilution of the inoculum (Sharma et al. (1980). However, there is hardly any information in the literature relating to the effect of inoculum size of *A. flavus* on aflatoxin production in liquid culture.

A novel approach to control fungal contamination of stored maize product is by combination treatment of moist heat (administered under high, >85% R.H. conditions) and gamma irradiation. This enables one to use a much lower dose in combination with mild heat treatment to achieve a very high level of inactivation of fungal spores (Odamtten et al., 1980a). The purpose of the present study is to investigate the influence of varying spore inoculum size on the production of aflatoxin B_1 by *A. flavus* NRRL 5906 into maize meal medium prior to heating of spores, after heating and its combination with gamma irradiation. This information B_1 production by irradiated fungal spores of *A. flavus* a phenomenon reported by some earlier workers (e.g. Applegate and Chipley, 1974). This study can also provide preliminary data on whether cereal grains treated by heat and gamma radiation might lead to enhanced aflatoxin B_1 formation by *A. flavus* or not.

5.2 Materials and methods

The stock culture of *Aspergillus flavus* NRRL 5906 was provided by Ko Swan Djien, Laboratory of Food Microbiology and Hygiene, Food Science Department, Agricultural University, The Netherlands. The fungus was maintained on maize meal agar (200 g blended maize, strained and made up to 1 litre with distilled water, 20 g plain agar added).

5.2.1 Treatment of dry spores of *A. flavus*

The spores were harvested dry from the sterilized (121 °C for 20 min.) cracked maize medium (200 g moistened cracked maize in 500 ml Erlenmeyer flask). The seven days old spores were separated from the maize medium by sieving with a plastic mesh and 0.1 g weight of spores transferred into sterile petri dishes.

The spore samples were unheated (20 °C) or heat treated at 60 °C for 30 min. in a heat-treatment chamber described by Odamtten et al. (1980b) that enabled us to expose the spores, during the heat treatment, to either a low humidity (<45% R.H.) ambient conditions or high humidity (>85% R.H.) ambient conditions. The lids of the petri dishes were excentrically placed on the bottom to allow maximum influx of moist or dry heat. The heated-treated spores were irradiated within 30 min. after the heat treatment in order not to lose the synergistic effect. After irradiation in the dry state, the spores were washed into suspension using 30 ml sterile 2% Tween-80 solution. An aliquot of 1.0 ml from the stock solution and the serially diluted stock $1:10^6$, suspension of spores (of known concentration) from each treatment combination was used in inoculating 250 ml Erlenmeyer flasks containing 25 ml of liquid medium, either maize grains, strained and made up to 1 litre with distilled water and sterilized twice at 121 $^{\circ}C$ for 20 min.) or ammended maize meal broth, AMMB (200 g blended maize grains, strained and made up to 1 litre with distilled water, 20 g peptone, 20 g glucose were added). When solid medium was required 20 g agar was added to 1 litre liquid medium. The pH of the cultures was determined by Marius pH meter type 52A.

5.2.2 Radiation treatment

The irradiation was carried out not more than 30 min. after the heat treatment (to ensure that the synergistic effect was not lost). The dose rate was 5.5 Krad/min. (0.06 KGy min^{-1}) and the absorbed dose was checked by clear perspex Dosimeters (HMP, 1.0 mm). Doses applied were 0.0, 3.5, and 4.0 KGy respectively.

5.2.3 Assessment of initial survivors and effect of serial dilution of stock suspension on aflatoxin B_1 formation

Dilutions were carried out of 1.0 ml of stock suspension of the untreated (control) and heat-treated spores (under both low, <45% R.H. and high, >85% R.H. ambient humidity conditions) up to $1:10^6$ and were plated out on maize meal agar (MMA) and ammended maize meal agar (AMMA). The number of colonies were counted after 3 days incubation at 28 $^{\circ}C$ from which the colony forming units per gm c.f.u.g^{-1} were calculated. Erlenmeyer flasks (250 ml) containing 25 ml AMMB were inoculated with 1.0 ml spore suspension of each dilution level up to $1:10^5$ stock suspension. There were five replicates for each dilution level. Dry weight of mycelium and amount of aflatoxin B_1 released by the mycelia into the flasks were determined, as detailed below, after 4 days incubation at 28 $^{\circ}C$.

5.2.4 Effect of varying inoculum size of control, heated and combined treated spores onaflatoxin B_1 production

This was essentially similar to the above experiment but differed in that the actual viable inoculum size of spores of *A. flavus* NRRL 5906 before heat

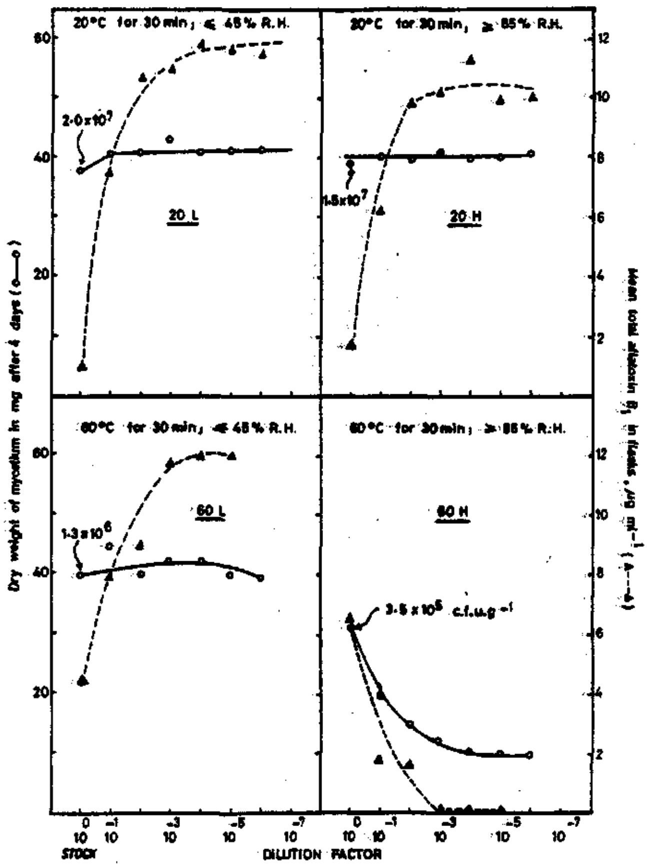


Fig. 1. Relationship between inoculum size (by serial dilution), vegetative growth and aflatoxin B_1 production by *A. flavus* NRRL 5906 spores incubated in AMMB for 4 days at 28 °C.

treatment were determined by both the dilution pour plate technique and also confirmed by spore density estimation using a Hawksley Cristalite B.S. 748 Haemacytometer (Hawksley and Sons Limited, Lancing Sussex, England). Viable spores after combination treatment of heat and gamma irradiation was ascertained by the spore count after 3 days incubation at 28 ^{o}C on AMMA and MMA and c.f.u.g^{-1} sample were thereafter calculated.

Erlenmeyer flasks containing either 25 ml of double-autoclaved MMB or AMMB were inoculated with 1.0 ml spore suspension of the pre-determined inoculum levels of spore concentrations (control, heat-treated and combined treatment of heat and gamma radiation). The conical flasks were incubated at 28 ^{o}C for 4 and 8 days respectively after which the dry weights of mycelium and total amount of aflatoxin B_1 produced were determined. Flasks containing maize meal broth, MMB were autoclaved twice to induce formation of higher levels of toxin (Odamtten et al., 1980c). There were seven replicates for each treatment and inoculum size used.

5.2.5 Assessment of dry weights of cultures

After the pre-determined incubation periods of 4 and 8 days respectively, growth in the liquid medium was assessed by estimating the dry weight of the harvested mycelium. Mycelium collected on a previously weighed and dried Ederol filter paper was dried at 75 ^{o}C for 24 h and then reweighed after cooling in a desiccator. The culture filtrates were retained for pH determination and extraction of aflatoxin.

5.2.6 Extraction of aflatoxins

We used a modification of the method of KO (1974). To estimate the amount of toxin released by mycelium into culture filtrates, an aliquot of 50 ml chloroform was added to each of four flasks (from which mycelium had been harvested). The aflatoxin extraction from the chloroform layer was enhanced by shaking at 100 rev. min^{-1} in a Gallenkamp shaker for 30 min. The chloroform extract phase was simply separated by means of a separating funnel. The chloroform extract containing the aflatoxins was evaporated to dryness using Rotavapor(R) vacuum rotation evaporator (Buchi, Switzerland). The dried residues were redissolved in 5.0 ml chloroform and kept in 250 ml Erlenmeyer flasks covered with cotton, wool and aluminium foil and then kept at 2 ^{o}C for one night or more since aflatoxins content determination of the culture filtrate by Thin Layer Chromatography (TLC) could not be completed on the same day.

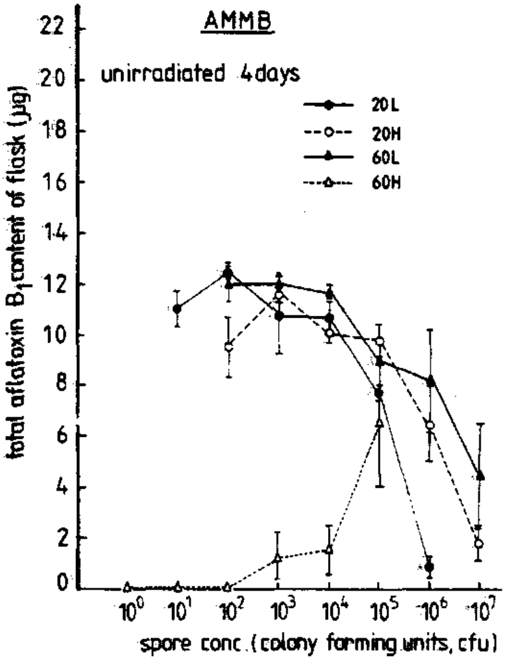


Fig. 2. Effect of spore density of *Aspergillus flavus* and ambient humidity during heat treatment on the production of aflatoxin B_1 in culture flasks containing ammended maize meal broth, AMMB incubated at 28 °C for 4 days. L - low humidity, $\leq 45\%$ R.H.; H - high humidity, $\geq 85\%$ R.H. (Note the inverse relationship between high spore density and low aflatoxin B_1 production.)

Mycelium of toxin producing fungi usually retain some of the toxin inside the cytoplasm of the mycelium depending on incubation period and other environmental conditions. To estimate the total amount of aflatoxin formed by *A. flavus* the mycelium and culture filtrate in three flasks were homogenised in an MSE Blendor and then washed with 75 ml of chloroform. The flasks containing the homogenised mycelium, culture filtrate and chloroform were shaken at 100 rev. min^{-1} for 30 min. and then subsequently treated in the same way as described above to obtain dried residue of filtrate and dissolved in 5.0 ml chloroform.

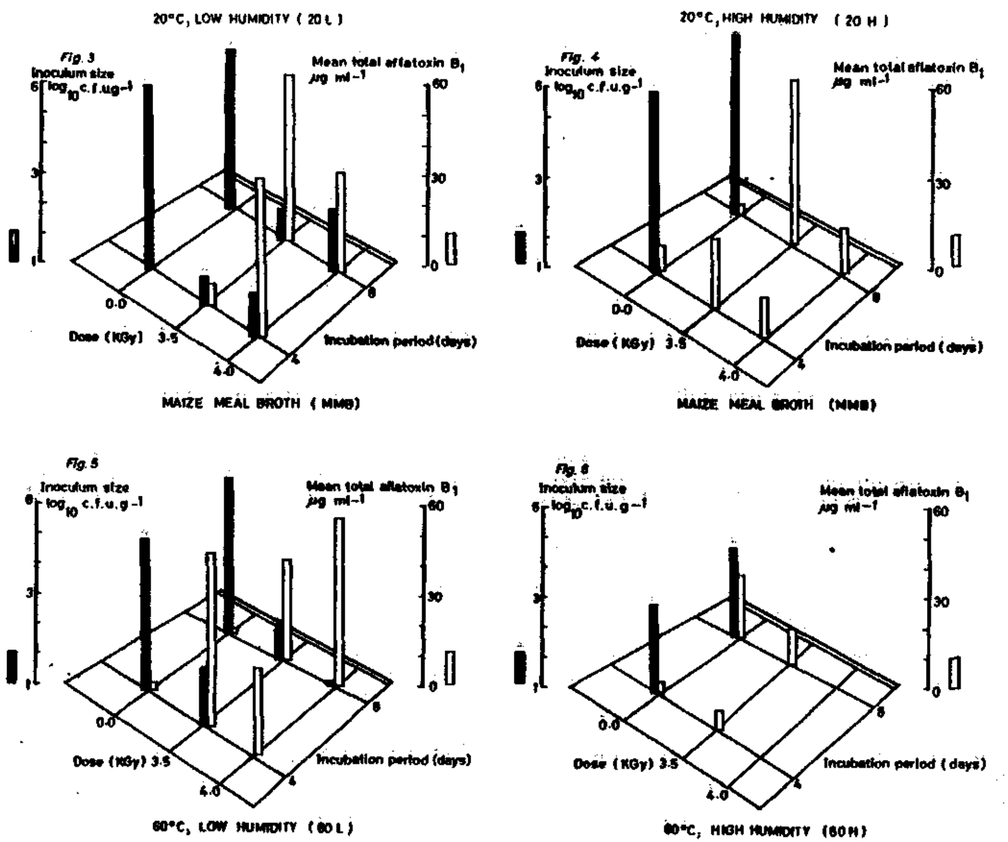
5.2.7 Quantitative determination of levels aflatoxin formed

Quantitative estimation of aflatoxin B_1 was carried out by using Thin Layer Chromatographic, TLC Technique. Samples were spotted on MN-Kieselgel G-HR plates (Machery, Nagel & Co. Duren W. Germany) using a Desaga Microdoser. Acetone:chloroform (1:9) was used as developing solvent. The intensity of the fluorescing spots was measured with a vitatron TLD-100 Densitometer (Vitatron Scientific Instrument, Dieren, The Netherlands) equipped with an integrator recorder. Amounts of aflatoxin B_1 formed were calculated by comparison of the integration units between the peak areas of the spots of the unknown sample and that of the known standard spots on the sample plate.

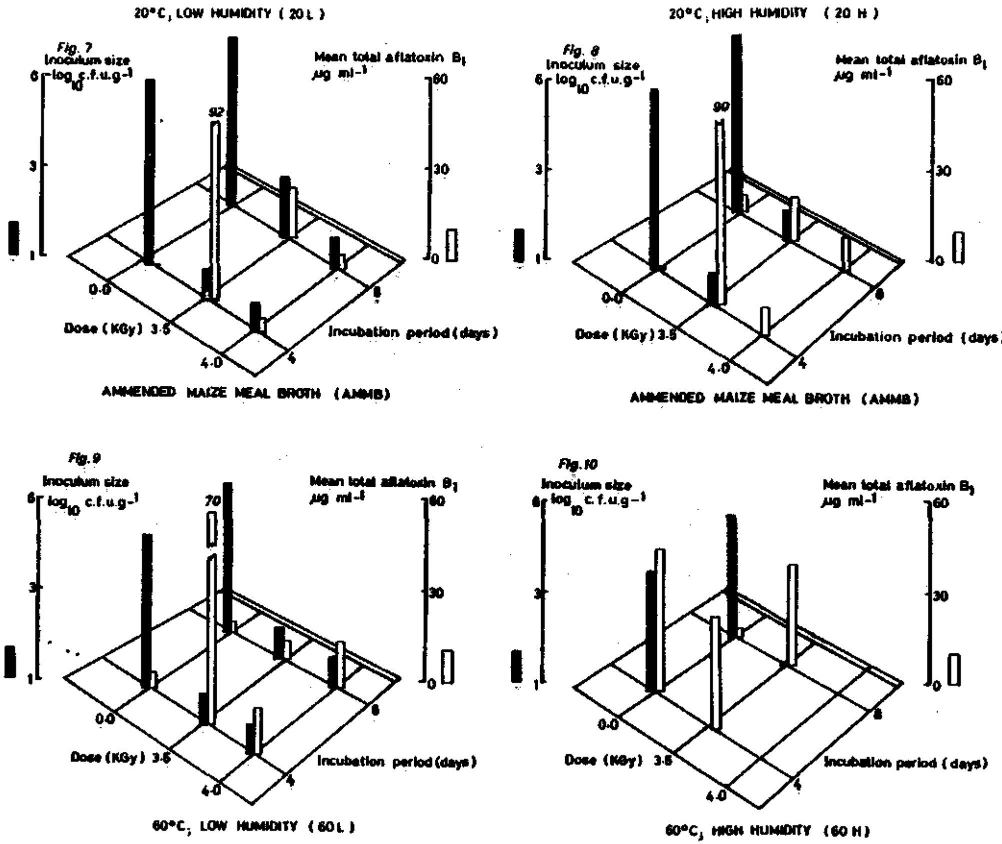
5.3 Results

5.3.1 Effect of the serial dilution of spore suspension on mycelial dry weight and aflatoxin B_1 formation

The results of the effect of serial dilution of spores of *A. flavus* NRRL 5906 on dry matter production and aflatoxin B_1 formation are summarised in Figs 1 and 2. After 4 days incubation at 28 ^{o}C in AMMB, dry matter production was independent of the inoculum size used in oculating flasks because serial dilution by 4-5 log cycles did not significantly change the dry weight of mycelium harvest from the flask containing unheated control spores (20 L, 20 H) and the heat-treated spores under low humidity conditions (60 L). However, a 2 log cycles reduction, 1:10^2 in spores that had been heat-treated (60 ^{o}C for 30 min.) under high humidity (>85% R.H.) conditions 60 H, resulted in the reduction of the mycelial dry weight by more than two-thirds (Fig. 1). A 3-4 log cycles reduction in inoculum size by serial dilution resulted in a twelve-fold increase in aflatoxin B_1 formed by control 20 L) spores; ten-fold increase in toxin formed by control (20 H) spores and a three-fold increase in aflatoxin B_1



Figs 3-6. Effect of inoculum size of *A. flavus* spores on aflatoxin B_1 production in maize meal broth (MMB) before and after combination treatment of heat and gamma irradiation. Control: 20 °C; L - low humidity treatment, ≤45% R.H.; H - high humidity, ≥85% R.H.



Figs 7-10. Effect of inoculum size of *A. flavus* spores on aflatoxin B_1 production in ammended maize meal broth, AMMB after combination treatment of heat and gamma irradiation.

produced by heat-treated spores under low-humidity (<45% R.H.) conditions (60 L). Interestingly, no toxin was formed in flasks containing spores heat-treated under high ambient humidity (>85% R.H.) conditions (60 H) and serially diluted by 3 log cycles. Generally high inoculum level of spores yielded the least amount of aflatoxin B_1 i.e. toxin formation was inversely related to the inoculum size (Fig. 2). When no toxin was formed in flasks (in high inoculum size conditions) the mycelium remained white or yellowish and culture filtrate was reddish in colour but reduction in inoculum size via serial dilution, heating, radiation or combination of heat and radiation resulted in green cultures with abundant spores and culture filtrates were yellow in colour.

5.3.2 Influence of inoculum size on the formation of aflatoxins by control and combined-treated spores

Figures 3-6 summarise the effect of radiation treatment and its combination with heat on the production of aflatoxin B_1 by *A. flavus* spores cultured in double autoclaved MMB. The effect is similar to what existed with the unheated control spores in Fig. 1. High inoculum size of spores yielded the least aflatoxin B_1. Reduction of the inoculum size by 3-5 log cycles via 3.5 KGy enhanced that aflatoxin B_1 formed by dry heat-treated spores (60 L) fifty-fold but nearly doubled ($1\frac{1}{2}$ times) aflatoxin B_1 formed by spores that had been moist heat-treated under high humidity conditions prior to applying 3.5 KGy. No aflatoxin B_1 was formed by spores that had been heat-treated under high humidity conditions prior to applying 4.0 KGy.

Figures 7-10 summarises the effect of radiation treatments and its combination with heat on the formation of aflatoxin B_1 in flasks containing AMMB. Results are similar to what existed in double autoclaved MMB except that greater amounts of toxin were formed in AMMB than in MMB and the mycelial dry weight were 3-8 times greater than that in MMB. Again, combination treatment of moist heat (60 ^{o}C for 30 min., applied under high humidity conditions, >85% R.H.) and 4.0 KGy of gamma radiation completely prevented aflatoxin B_1 formation by the spores in the culture medium. Aflatoxins B_2, G_1 and G_2 were formed along side B_1. But when aflatoxin B_1 formation is attenuated by a combination of moist heat and 4.0 KGy of gamma radiation, aflatoxins B_2, G_1 and G_2 could not be detected either.

5.4 Discussion

The data from these studies indicate that total growth of unirradiated spores was independent of inoculum size in same volume of medium. The only exception was the spores that had been heat-treated (60 ^{o}C for 30 min.) under high ambient (>85% R.H.) humidity conditions where serial dilution reduced the dry weight by two-thirds. Moist heat therefore has an intrinsic lethality. The stress of heat on essential biological molecules of the spore is augmented by high humidity conditions. Heat is also transferred more readily in wet air (Hawker et al., 1952). It is well documented that in addition to temperature and duration of treatment, humidity strikingly affects lethality of heat applied. The gradual increase in aflatoxin B_1 production was related to the decrease in inoculum size by serial dilution. Moist heat treatment could reduce the population of spores exponentially and affected their toxigenic potential after 3 log cycles reduction in population.

The growth of fungi is restricted to the apical region of the mycelium and the apex is constantly supplied with nutrients from the rest of the mycelium for cell multiplication and branching (Burnett, 1968). Studies by Jinks (1969) and Sharma et al. (1980) suggest that aflatoxin production in a medium might be associated with mycelial branching and differentiation. Hyphal fusion, common in the Deuteromyces (Park & Robinson, 1966; Raper, 1952) will be faster in a large population of spores and faster release of certain substances may also influence the lateral branching (Burnett, 1968; Gottlieb, 1971; Park and Robinson, 1966). With smaller populations, these limitations are reduced to afford more lateral branching or secondary mycelial growth, which may in turn result in higher yield of aflatoxin in these cultures. Indeed, we found that diluted spores in cultures produced the highest amount of aflatoxin B_1 and that cultures developing from dilute inocula branched and sporulated profusely and faster after 4 days incubation in AMMB or MMB. There was no sporulation in control unheated spores developing from large inocula suggesting the involvement of mycelial differentiation in aflatoxin B_1 production in *A. flavus* NRRL 5906. This observation contrasts the findings of Sharma et al (1980) who reported that cultures developing from dilute inocula of *A. parasiticus* NRRL 3145 hardly sporulated even after prolonged incubation, whereas sporulation was much faster and profuse in cultures developing from large inocula. Our findings confirm that of Detroy et al. (1971), that aflatoxin synthesis occurs during the period of intense sporulation of the fungus and that good aflatoxin production correlated with yellowing to greening of the mycelium and the medium. In flasks which failed to yield aflatoxin B_1, the mycelium remained white and the medium was either clear or slightly reddish (Mateles and Adye, 1965).

Irradiation alone (3.5 KGy) or its combination with heating reduced the inoculum size by 3-5 log cycles which resulted in enhanced aflatoxin B_1 synthesis. The effect was similar to what existed by serial dilution of the untreated large inoculum of spores. Thus irradiation may not have any direct effect on aflatoxin-producing ability but on the size of inoculum in relation to the viable spores. However, when heat-treatment is administered (60 ^{o}C for 30 min.) under moist humid conditions (>85% R.H.) in combination with 4.0 KGy of gamma radiation, *A. flavus* NRRL 5906 growth and aflatoxins formation is completely impaired. The production of aflatoxin by *A. flavus* NRRL 3145 was reported to be monophasic in the cultures exposed to gamma irradiation by Applegate and Chipley (1977). We did not trace aflatoxin-producing pattern of *A. flavus* NRRL 5906 beyond 8 days. However, maximum aflatoxin B_1 formation by treated spores was generally 4 days in AMMB and 8 days in MMB. Greater amount of toxin was formed in AMMB than in MMB, presumably for two reasons: (i) the faster growth of spores in AMMB, and (ii) the presence of 2% peptone and 2% glusoce in AMMB which are required in the synthetic pathway for aflatoxin biosynthesis.

We conclude that a combination treatment of moist heat, 60 ^{o}C for 30 min. (>85% R.H. ambient) and 4.0 KGy of gamma irradiation can prevent both growth and aflatoxin production by *A. flavus* NRRL 5906. Aflatoxin B_1 formation is inversely related to the inoculum size of *A. flavus* NRRL 5906. The apparent enhancement of aflatoxin B_1 formation by a combination of heating and 3.5 KGy is a direct result of reduction in inoculum size of 3-4 log cycles. Thus irradiation and its combination with heat may not have any direct effect on aflatoxin-producing ability but on the size of the inoculum in relation to viable spores. This effect is similar to what existed with serial dilution of untreated and irradiation spores. The effect of this combination treatment of moist heat (60 ^{o}C for 30 min.) and irradiation in preventing aflatoxin in maize grains artificially inoculated with *A. flavus* is examined in a subsequent paper.

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6. STUDIES ON THE COMPARATIVE EFFICIENCY OF WOVEN POLYPROPYLENE AND JUTE SACS AS POSSIBLE PACKAGING MATERIAL FOR PRE- AND POST-IRRADIATION STORAGE OF SOME CEREAL GRAINS

G.T. Odamtten.

Abstract

The importance of using the correct storage bag for extending the shelf-life of stored food, is the primary concern of the producer and buyer alike. Studies reported here were carried out to compare the efficiency of two sacs (jute and woven polypropylene) in preserving grain quality from fungal deterioration so that it could be used for pre- and post-irradiation storage of cereal grains. After one month's storage and using Student's "t" test, the difference in viability (% germination) of maize grains in both sacs was generally not statistically significant ($P = 0.01$ or 0.05) irrespective of where sacs were placed in the warehouse. However, after 2-4 months storage, the efficiency of woven polypropylene sacs became apparent when the viability of maize grains kept in such sacs was statistically higher ($P = 0.01$ or 0.05) than that kept in jute sacs. Correspondingly, there was a lower fungal contamination (initial 1.2×10^2 c.f.u.g^{-1} to 7.6×10^2 c.f.u.g^{-1} in 4 months) on the maize grains kept in woven polypropylene sacs than on maize grains stored in jute sacs (initial 1.2×10^2 c.f.u.g^{-1} to 7.0×10^4 c.f.u.g^{-1} in 4 months). Rice grains kept in the two different sacs under investigation did not differ significantly ($P = 0.01$ or 0.05) in their fungal contamination after 1, 2 and 4 months storage periods. But rice grains kept in jute sacs were heavily infested by *Corcyra cephalonica* (Stantion), the larvae constructing silken tunnels in which they lived, causing aggregation of the rice grains. *Ephestia cautella* (Walker) infested rice in jute sacs to a lesser extent.

Certain fungi were persistently isolated in the weekly air sampling for mycoflora in the warehouse, despite the sporadic aerial spraying with Actellic 25$^{(R)}$. These were *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme, Mucor pusillus, Penicillium verrucosum* var. *cyclopium* and *Rhizopus oryzae*. Fumigants, therefore, do not kill fungi. These listed fungi also infected greater percentage of maize grains kept in both jute sacs and woven polypropy-

lene sacs. New species of fungi recorded for the first time on maize in Ghana are *Aspergillus glaucus, Aspergillus japonicus, Fusarium nivale, F. oxysporium, F. solani, Mucor pusillus* and *Mycelia sterilia*. The practical importance of these findings are discussed and future work suggested.

6.1 Introduction

Sanitation requirements for preventing food infection and food poisoning are contained in food legislation throughout the world (1). The objective of choosing a proper food packaging material is to provide safety for food consumed by the general population.

In Ghana, the use of jute sacs for storing food items such as cowpeas, rice, groundnut, maize, millet, sorghum and cocoa is well known and the attendant loss of food owing to insects and fungi is estimated at over 30% of the annual harvest (2). Jute sacs allow influx and efflux of air and moisture and the penetration of insecticides during routine prophylactic applications. In addition jute sacs have low tear resistance, low bursting strength, are water permeable and have high water vapour transmission rate. Despite these mishaps, the use of these sacs is still popular because it is cheaper than the suggested substitutes such as woven polypropylene sacs.

The first commercial use of polypropylene as a sac material was in 1958 for packaging of 25 kg quantities of fertilizer and polyethylene resins (3). From then on, progress in the use of heavy-duty polypropylene sacs was fairly rapid in England, Canada, United States of America and South Africa, mainly for fertilizer packaging. Within the European Economic Community (EEC) and USA, the use of woven polypropylene sacs, which are much lighter and stronger than jute sacs have become the rule rather than an exception, for packing food items such as maize, rice, etc. which are sent to Africa as food aid.

Among the factors that are of major concern in packaging problems connected with radiation preservation of food are (i) the capacity of the container to preserve the irradiated food during handling and storage (ii) imperviousness of the container and the container material to entry of spoilage organisms (iii) the capacity of the container material to accept, without undue injury to its chemical composition and physical structure, periods of exposure to gamma rays of high energy electrons and (iv) the capacity of the container material to submit to radiation without imparting off-odours or flavours to the contents of the food package (4). To assess a sac for its suitability for packing of irradiated cereal grains as spelt out in (i) and (ii) the major types of losses

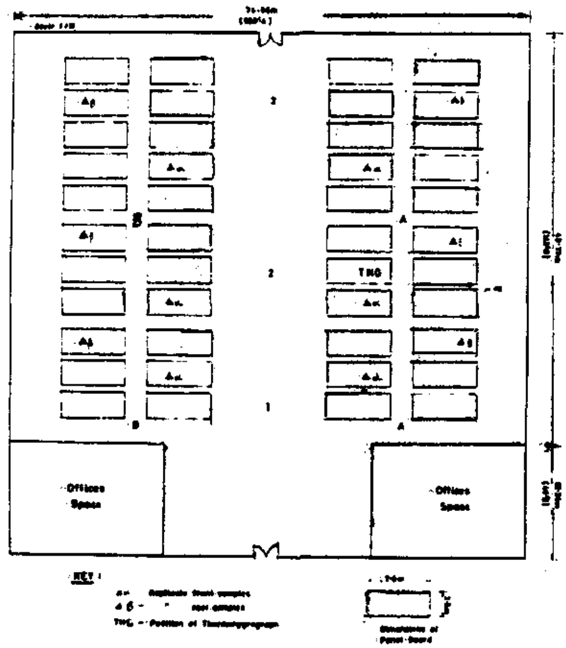

Fig. 1. Photograph showing the layout of the stored maize grains within the Grains Warehouse. The positions of replicate samples on top of the stacks are indicated by shaded triangle (▲).

caused by fungi contaminating stored cereal grains should be examined; namely (a) decrease in germination, (b) discolouration of part or all of the grain or kernel, (c) heating or mustiness, (d) various biochemical changes, (e) production of mycotoxins, and (f) loss in weight (5).

In recent studies (6, 7) maize grains and animal feed were artificially inoculated with *Aspergillus flavus* Link. and the samples were treated with a combination treatment of moist heat (60 ^{o}C for 30 min. under high humidity conditions ($\geq$85% R.H.) and gamma radiation of 4.0 KGy before being stored in woven polypropylene sacs. The samples which were stored at 28 ^{o}C and 80% R.H. for 4 months still had good keeping quality (i.e. no enterobacteriaceae, no fungal growth, no increase in free fatty acid, and no aflatoxin B_1 production). There is no information in the pertinent literature on comparative studies with jute sacs in this regard. Studies reported here were designed to provide information on the comparative ability of jute and woven polypropylene in keeping cereal grains (maize and rice) in good quality conditions during prolonged storage under practical field conditions.

6.2 Materials and methods

The maize grains and rice were provided by the Grains Warehousing Company, Tema. Each pallet board carried 6-9 jute sacs containing 50 kg maize and arranged horizontally. Twenty to twenty-one such sacs were stacked one on top of another. Twelve woven polypropylene sacs were then filled with 50 kg wt of maize grains and the sacs were randomly placed on top of the stacks in the positions indicated in Fig. 1. One woven polypropylene sac was placed adjacent to one of the jute sacs at each of the indicated locations. The Warehouse has a storage capacity for 75,000 tons.

The rice storage room was about one-quarter the size and capacity of the Warehouse where the maize grains were kept. Each pallet board again carried 6-9 jute sacs containing 50 kg of rice and arranged horizontally on the board. Twenty to twenty-one such sacs were stacked on top of one another on the pallet board. Four woven polypropylene sacs containing 50 kg wt fo rice were placed on four such stacks of jute sacs randomly selected in the Rice Warehouse.

6.2.1 Measurement of ambient Relative Humidity and Temperature

A thermohygrograph (Wilh. Lambrecht K.G., Göttingen), THG, was placed on top of the stacks of sacs in Bay 8. This automatically recorded simultaneously

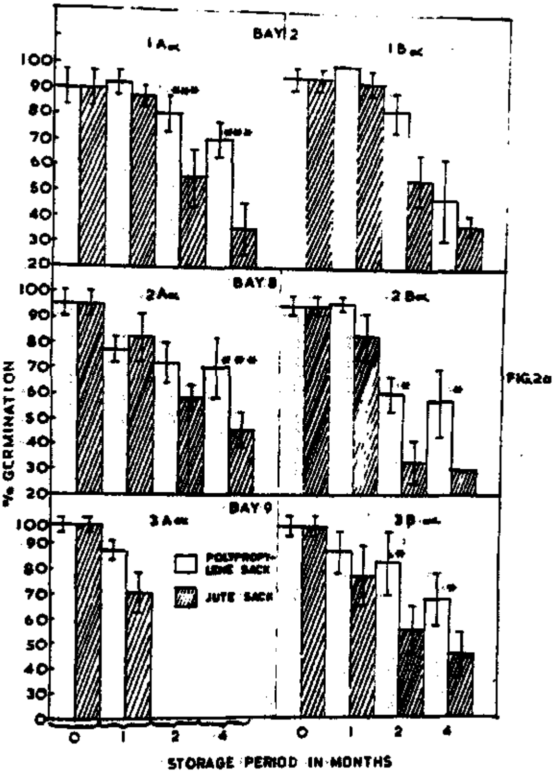

Fig. 2a. Changes in viability (% germination) of maize grains kept in the positions in two different indicated storage sacs kept for 1, 2 and 4 months.
(Data represents average results of 50 petri plates each containing 10 grains.)

* P = 0.05
*** P = 0.01
Unmarked - not significantly different.

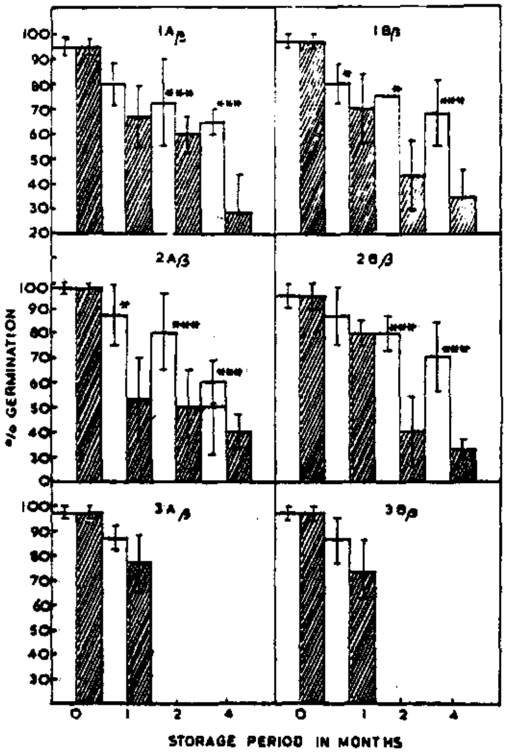

FIG.2b.

Fig. 2b.
Changes in viability (% Germination) of maize grains kept in the positions in two different indicated storage sacs kept for 1, 2 and 4 months. (Data represents average results of 50 petri plates each containing 1C grains).

* P = 0.05
*** P = 0.01
Unmarked - Not statistically different.

the daily ambient relative humidity (R.H.) and temperature on a weekly chart. The chart was changed every week. At the end of every month, the R.H. and temperature within the two different storage bags (jute and woven polypropylene) were ascertained, using an Electronic Thermometer/Hygrometer Model HT-1 (Delmhorst Instrument Co., Boonton, New Jersey, USA) designed to measure temperature and relative humidity simultaneously on two metres caliberated for direct reading. The instrument is provided with three aluminium probe rods, each one metre long with a terminal SN-1 Sensor which was inserted into the sacs. Six readings were taken at one minute intervals for each bag and data obtained represent average of six successive readings.

6.2.1 Sampling of air mycoflora

Sterile petri plates containing oxytetracycline glucose yeast extract agar (OGYE) were momentarily exposed (for 5 min.) in the Warehouse at Bay 3 (1Aα, 1Aβ; 1Bα, 1Bβ); Bay 8 (2Aα, 2Aβ; 2Bα, 2Bβ) and Bay 9 (3Aα, 3Aβ; 3Bα, 3Bβ). Sixteen replicate petri plates (i.e. four petri plates in each of the α and β positions) were exposed at each Bay for sixteen weeks. During the 7th, 11th, 14th and 16th weeks samplings, petri plates containing Sabouraud's Agar were included (four petri plates in each of the α and β positions in Fig. 1) to see if any differences exist in the kinds of fungi and no. of fungal colonies isolated on the two media (OGYE, and Sabouraud's Agar). The petri dishes were incubated at 28 ^{o}C until colonies appeared in 3-5 days. The total no. of colonies and the species of fungi isolated were recorded at each weekly interval. There was prophylactic application of insecticide Actellic 25 $^{(R)}$ during the sampling of the air mycoflora. These periods were noted and taken into consideration during analysis of results.

6.2.3 Determination of viability of grains

The maize grains were sampled monthly for 1, 2 and four months. At each sampling period 500 g of grains were taken from the top, middle and bottom of the jute and woven polypropylene sacs and the samples from each sac were then pooled together. There were twelve pooled samples for each type of storage sac. The mycoflora on the grains and their percentage germination (viability) were assessed using the blotter test of Tempe (8) and Limonard (9). Ten grains from either jute sac of woven polypropylene sac were placed on sterile Whatman's filter paper in 9 cm petri dishes. There were 50 replicates (500 grains) for each of the 12 sample grains from jute sacs and woven polypropylene sacs res-

Table 1. Record of fungal contamination on maize grains kept in woven polypropylene sacs for the indicated periods.

Location	Replicates	Storage period in months			
		0 (Initial)	1	2	4
Bay 3	1Aα	1.2×10^2	5.4×10^1	1.6×10^2	2.8×10^2
	1Aβ		7.8×10^1	3.0×10^2	3.2×10^2
	1Bα		1.2×10^2	6.4×10^2	2.8×10^2
	1Bβ		8.6×10^1	1.4×10^2	2.6×10^2
Bay 8	2Aα	1.2×10^2	4.2×10^1	2.8×10^2	3.5×10^2
	2Aβ		3.4×10^1	5.0×10^2	2.5×10^2
	2Bα		2.2×10^1	5.8×10^1	5.2×10^2
	2Bβ		6.5×10^1	2.1×10^2	7.6×10^2
Bay 9	3Aα	1.2×10^2	2.8×10^1	-	-
	3Aβ		3.7×10^1	-	-
	3Bα		4.2×10^1	6.4×10^1	8.0×10^1
	3Bβ		5.3×10^1	$1.4\times10^{2*}$	$3.2\times10^{2*}$

*Microbial count determined from samples in a different bag (for 3Bβ only); original bags having been inadvertently carried away. Data quoted are in colony forming units per gm sample ($c.f.u.g^{-1}$).

Table 2. Record of fungal contamination on maize grains kept in jute sacs for the indicated periods.

Location	Replicates	Storage period in months			
		0 (Initial)	1	2	4
Bay 3	1Aα	$1.2x10^2$	$7.8x10^2$	$2.0x10^4$	$2.0x10^4$
	1Aβ		$1.2x10^2$	$6.2x10^3$	$4.4x10^3$
	1Bα		$4.0x10^2$	$1.6x10^3$	$3.6x10^4$
	1Bβ		$1.1x10^3$	$6.8x10^3$	$6.6x10^3$
Bay 8	2Aα	$1.2x10^2$	$5.2x10^2$	$3.6x10^4$	$2.8x10^4$
	2Aβ		$1.1x10^2$	$6.4x10^4$	$5.2x10^4$
	2Bα		$1.4x10^3$	$1.4x10^3$	$1.4x10^4$
	2Bβ		$1.7x10^2$	$1.6x10^3$	$7.0x10^4$
Bay 9	3Aα	$1.2x10^2$	$1.2x10^3$	-	-
	3Aβ		$2.2x10^2$	-	-
	3Bα		$1.1x10^2$	$4.2x10^3$	$5.4x10^4$
	3Bβ		$1.3x10^2$	$1.8x10^{2*}$	$2.4x10^{4*}$

*Microbial count determined from samples in a different bag (for 3Bβ only); original bags having been carried away. Data quoted are in colony forming units per gm sample (c.f.u. g^{-1}).

Table 3. Record of fungal contamination of rice grains kept in named sacs for the indicated periods.

Location	Replicates	Storage period in months			
		0	1	2	4
Rice Room (Jute sac)	1A	7.9×10^1	7.6×10^1	7.4×10^1	7.6×10^1*
	2A		3.2×10^1	5.4×10^2	8.2×10^1
Rice Room (Woven poly-propylene sac)	1B	7.9×10^1	1.6×10^1	6.8×10^1	1.9×10^1*
	2B		4.0×10^1	7.2×10^1	7.6×10^1

*Fungal contamination not statistically different at both, $P = 0.01$ and $P = 0.05$ level of significance.

pectively and the plates were incubated for up to 14 days (10) at 28±3 °C. The following monthly quantitative assessments were made for the blotter test:

(a) the percentage of grains germinating. Any grain producing roots or coleoptile was considered to have germinated;

(b) the percentage of grains infected with a particular fungus species.

6.2.4 Procedure for preparation of homogenate of spores for microbial load determination of maize grains

Exactly 25 g of the samples (maize or rice grains) were weighed into sterile 250 ml Erlenmeyer flasks containing 100 ml sterile 0.1% peptone solution as dilution blanks. The contents were shaken in a Gallenkamp Orbital Shaker at 140 $rev.min^{-1}$ for 30 min. The resulting homogenate was regarded as initial stock spore suspension from which dilution series were prepared. The plates were incubated at 28 °C for 3 days after which the colonies appearing were counted and hence the colony forming units (c.f.u.) per gram sample were determined.

6.2.5 Statistical analysis

Data, where appropriate were analysed using Student's "t" test and the results were tested for significance at $P = 0.01$ or 0.05 levels of significance.

6.3 Results

After one month's storage and using Student's "t" test, the difference in viability (% germination of maize grains in both jute sacs and woven polypropylene was generally not statistically significant ($P = 0.01$ or 0.05) irrespective of where the sacs were placed in the Warehouse (Fig. 1). The only exception for the first month's germination data was at site 2A where the difference in the % germination of maize grains kept in woven polypropylene sac was significantly higher ($P = 0.05$) than grains stored in jute sac (Figs 2a and 2b). After 2-4 months storage period, the efficiency of woven polypropylene sacs became apparent, when the viability of maize grains kept in such sacs were statistically higher ($P = 0.01$ or 0.05) than those stored in jute sacs (Figs 2a and 2b). Correspondingly, there was a lower fungal contamination (initial, 1.2×10^2 $c.f.u.g^{-1}$ to 7.6×10^2 $c.f.u.g^{-1}$ in 4 months) on the maize grains kept in woven polypropylene sacs than on maize grains stored in jute sacs (initial, 1.2×10^2 $c.f.u.g^{-1}$ to 7.0×10^4 $c.f.u.g^{-1}$ in 4 months (Tables 1 and 2). Rice

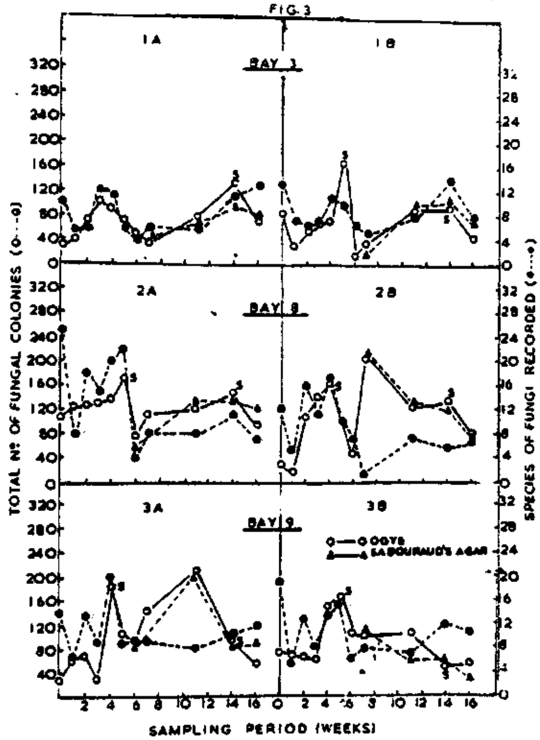

Fig. 3. Graph showing occurrence of micoflora in the Warehouse illustrated in Fig. 1. S represents spraying with insecticide.

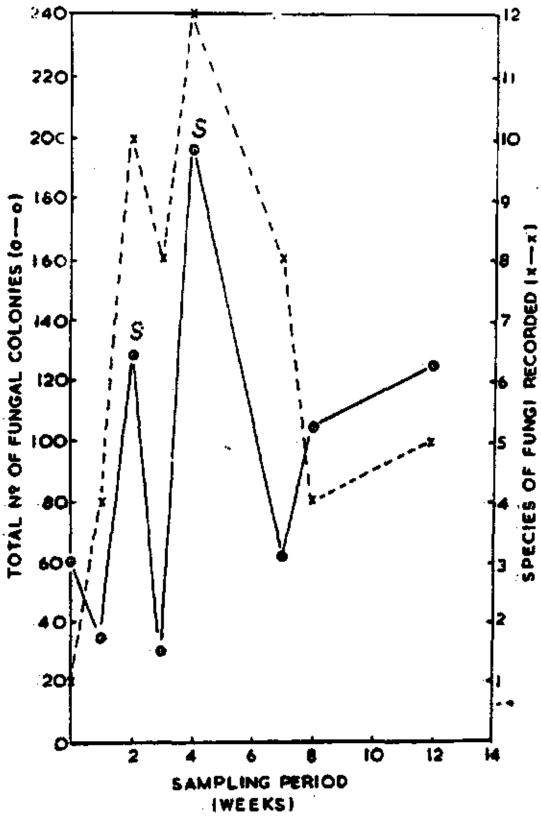

Fig. 4 Graph showing occurrence of mycoflora in the Rice Storage Room for the indicated periods.

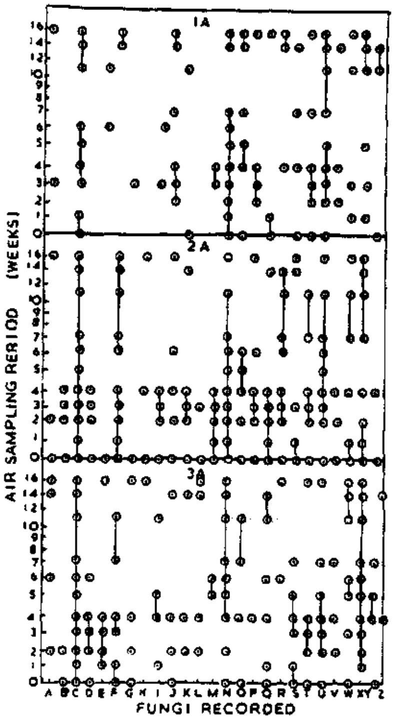

Fig. 5a Scatter graph showing occurrence of fungi at the indicated positions (1A, 2A, 3A) in the Grains Warehouse (Fig. 1) (Read graph vertically along each indicated fungus)

(a) O———O, Unbroken lines; represent consecutive week(s) isolation of indicated fungus.

(b) O, isolated spots; sporadic isolation of indicated fungus.

__FUNGI RECORDED:__

A. __Aspergillus candidus__
B. __A. carneus__
C. __A. flavus__
D. __A. glaucus__
E. __A. japonicus__
F. __A. niger__
G. __A. ochraceus__
H. __A. tamarii__
I. __Aspergillus sp.__
J. __Cephalosporium acremonium__
K. __Curvularia lunata__
L. __C. semitectum__
M. __Drechslera maydis__
N. __Fusarium moniliforme__
O. __F. nivale__
P. __F. oxysporium__
Q. __Mucor pusillus__
R. __Mycelia sterilia__
S. __Neurospora sitophila__
T. __Penicillium digitatum__
U. __P. verrucosum var cyclopium__
V. __Penicillium sp.__
W. __Rhizoctonia solani__
X. __Rhizopus oryzae__
Y. __Trichoderma viride__
Z. __Paecilomyces varioli__

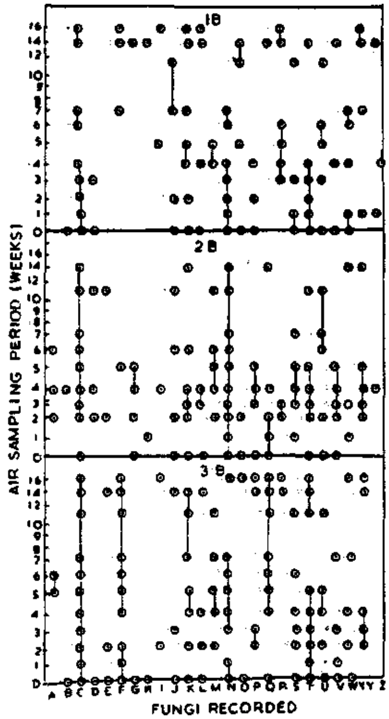

Fig. 5b Scatter graph showing occurrence of fungi at the indicated position (1B, 2B, 3B) in the Grains Warehouse (Fig. 1). (Read graph vertically along each indicated fungus)

(a) O——O Unbroken lines; represents consecutive week(s) isolation of the indicated fungus.

(b) O; isolated spots, sporadic isolation of indicated fungus.

FUNGI RECORDED:

A. Aspergillus candidus
B. A. carneus
C. A. flavus
D. A. glaucus
E. A. japonicus
F. A. niger
G. A. ochraceus
H. A. tamarii
I. Aspergillus sp.
J. Cephalosporium acremonium
K. Curvularia lunata
L. C. semitectum
M. Drechslera maydis
N. Fusarium moniliforme
O. F. nivale
P. F. oxysporium
Q. Mucor pusillus
R. Mycelia sterilia
S. Neurospora sitophila
T. Penicillium digitatum
U. P. verrucosum var cyclopium
V. Penicillium sp.
W. Rhizoctonia solani
X. Rhizopus oryzae
Y. Trichoderma viride
Z. Paecilomyces varioti.

grains (polished rice) kept in both jute and woven polypropylene sacs did not differ significantly ($P = 0.01$ or 0.05) in their fungal contamination after 1, 2, and 4 months storage periods (Table 3). However, rice grains kept in the jute sacs were, relatively, heavily infested by *Corcyra cephalonica* (Stantion), the larvae constructing silken tunnels in which they lived, causing aggregation of the rice grains. *Ephestia cautella* (Walker) infested rice in jute sacs to a lesser extent.

The record of total number of fungal colonies and species of fungi isolated in the aerial sampling in the Warehouse (Fig. 1) and Rice Storage Room are illustrated in Figs 3 and 4, respectively. Aerial prophylactic spraying with fumigant Actellic 25$^{(R)}$, which has been a routine practice in the Warehouse, could not be prevented and this exercise was always followed by a decline in the number of species of fungi isolated and also the number of fungal colonies. But this was followed by an increase in the air mycoflora in 2-4 weeks (Fig. 3) in the Warehouse where maize grains were kept and 1-3 weeks (Fig. 4) in the Rice Storage Room. Although aerial spraying with insecticides might kill insects, it did not completely eliminate fungal spores. Also, in between the stacks of jute sacs was fumigation preparation "Detia Gas Ex-B" (Phosphine gas) in bags (6x8 cm). This did not kill the fungal spores and they were isolated throughout the 4 months sampling period.

During the air mycoflora sampling period the species of fungi that were isolated were noted and their occurrence in the various selected section of the Warehouse (Fig. 1) was recorded (Figs 5a and 5b). In a similar manner, the fungal species that were isolated in the Rice Store Room are recorded in Fig. 6. Certain fungi were frequently isolated in the weekly air sampling for presence of mycoflora in the Warehouse. These were *Aspergillus flavus, Aspergillus niger, Fusarium moniliforme, Mucor pusillus, Penicillium verrucosum* var. *cyclopium* and *Rhizopus oryzae* (Figs 5a,b). These listed fungi also infected a greater percentage of maize grains kept in both jute and woven polypropylene sacs (Tables 4a-e). The fungi which predominated in the air mycoflora in the Rice Storage Room were *Aspergillus flavus, Aspergillus niger, Curvularia lunata, Fusarium moniliforme* and *Penicillium digitatum* (Fig. 6). Very few species of fungi were found on the rice grains when the microbiological load on them was determined. The moulds were *Curvularia lunata, Aspergillus niger* and *Fusarium moniliforme*, in order of abundance. Fungal species recorded for the first time on maize in Ghana, during this investigation, are *Aspergillus glaucus, Aspergillus japonicus, Fusarium oxysporium, Fusarium solani, Mucor pusillus* and *Mycelia sterilia*. There was no significant difference between OGYE and Sabouraud Agar as a

FIG. 6

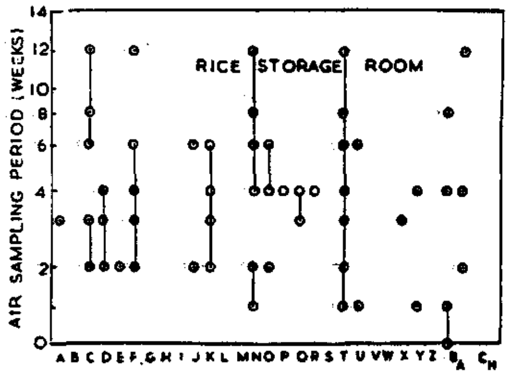

Fig. 6 Scatter graph showing occurrence of fungi in the Rice Store Room during a twelve week period. (Read graph vertically along each indicated fungus)

(a) O——O Unbroken lines; represent consecutive week(s) isolation of indicated fungus.

(b) O isolated spots; sporadic isolation of indicated fungus.

FUNGI RECORDED:

A. Aspergillus candidus
B. A. carneus
C. A. flavus
D. A. glaucus
E. A. japonicus
F. A. niger
G. A. ochraceus
H. A. tamarii
I. Aspergillus sp.
J. Cephalosporium acremonium
K. Curvularia lunata
L. C. semitectum
M. Drechslera maydis
N. Fusarium moniliforme
O. F. nivale
P. F. oxysporium
Q. Mucor pusillus
R. Mycelia sterilia
S. Neurospora sitophila
T. Penicillium digitatum
U. P. verrucosum var cyclopium
V. Penicillium sp.
W. Rhizoctonia solani
X. Rhizopus oryzae
Y. Trichoderma viride
Z. Paecilomyces varioti
B_A. Bacteria (unidentified)
CH. Cladosporium herbarum.

medium for isolating the mycoflora (Fig. 3).

Records of ambient temperatures and relative humidities taken simultaneously using a thermohygrograph placed in the Warehouse (Fig. 1) are represented in Fig. 7 (9th December - 29th December 1981), Fig. 8 (1st January - 26th January 1982), Fig. 9 (February 1982), Fig. 10 (3rd - 23rd March 1982), and Fig. 11 (24th March - 5th April 1982). There are large fluctuations in relative humidities (25-90% R.H.) and less so with temperature (25-40 $^{\circ}$C) records in the Warehouse. The lowest R.H. recorded was 25%, on the 5th of January, 1982, during the harmattan season (late December - early February). The lowest R.H. was always recorded at midday (12.00 hr GMT). The relative humidity in the Warehouse was high during the early part of the day (1.00-6.00 hr GMT) and thereafter decreased. A second high humidity was recorded during the period 18.00-24.00 hr GMT (midnight).

Conversely, the highest ambient temperature was always recorded at midday (12.00 hr GMT), with the lowest during the early part of the day and in the evening. The two communicating doors in Fig. 1 were opened during the day (07.30-16.30 hr GMT) and thereafter remained closed till the following day. These results indicate that Equilibrium Relative Humidity (E.R.H.) was not attained in the Warehouse during the entire storage period of 4 months. In contrast with this, a corresponding record of R.H. and temperature taken from our laboratory, where some of the maize grains and rice were stored for the period 24th March - 14th April 1982, showed that the R.H. and temperature remained fairly constant throughout the period (Fig. 12). Under such conditions. E.R.H. is attained in 12 days. The moisture content (m.c.) of grains depend on the storage humidity. The higher the storage humidity, the higher the equilibrium m.c. of grains. But the m.c. of maize grains does not increase appreciably at E.R.H. 80% and below, after 12 days storage period (10). If there are large diurnal fluctuations in ambient R.H. and temperature, such as obtained in the Warehouse during these investigations, m.c. of the grains will not reach equilibrium with the ambient R.H. The m.c. of the grains will be closely related to the prevailing R.H. at the time and day of sampling (Table 5). The m.c. of grains kept in jute sacs was higher than those stored in woven polypropylene sacs (Table 5).

Table 4a. Table showing species of fungi isolated from maize grains and their percentage on grains they contaminated in the indicated sacs.

Location code	Fungi recorded on grains	% Occurrence on grains kept in			
		Jute sacs for		Polypropylene sacs for	
		2 months	4 months	2 months	4 months
1Aα	*Aspergillus flavus* Link	47.5	27.5	40.0	55.0
	A. glaucus	-	-	2.5	-
	A. niger Van Tieghem	52.5	35.0	22.5	25.0
	A. ochraceus Wilhelm	7.5	-	-	2.5
	A. ustus Wehmer	-	-	2.5	-
	Drechslera maydis (Nisikado) Subraim & Jain	-	-	-	20.0
	Fusarium moniliforme Sheldon	100.0	22.5	82.5	75.0
	Fusarium oxysporium	-	-	-	20.0
	Neurospora sitophila Shear & Dodge	5.0	25.0	-	-
	Paecilomyces varioti Bain	-	2.5	-	-
	Penicillium verrucosum var. *cyclopium*	30.0	25.0	90.0	72.5
	Rhizoctonia solani Kuhn	5.0	-	25.0	-
	Rhizopus oryzae Went and Prinsen Geerling	100.0	17.5	7.5	32.5
1Aβ	*Aspergillus flavus* Link	40.0	27.5	52.5	47.5
	A. glaucus	2.5	7.5	5.0	12.5
	A. japonicus	-	2.5	-	-
	A. niger	15.0	27.5	17.5	22.5
	A. ochraceus	12.5	10.0	2.5	-
	A. ustus	2.5	-	5.0	2.5
	Drechslera maydis	7.5	-	12.5	-
	Caldosporium herbarum Link ex Fr.	-	-	2.5	-
	Curvularia lunata (Wakker) Boedjin	2.5	-	5.0	-
	Penicillium verrucosum var. *cyclopium*	17.5	20.0	30.0	77.5
	Rhizopus oryzae	10.0	12.5	5.0	50.0
	Fusarium moniliforme	15.0	27.5	57.5	30.0
	Fusarium oxysporium	2.5	-	7.5	-

Table 4b.

Location code	Fungi recorded on grains	% Occurrence on grains kept in			
		Jute sacs for		Polypropylene sacs for	
		2 months	4 months	2 months	4 months
2Aα	*Aspergillus flavus* Link	40.0	97.5	62.5	27.5
	A. glaucus	2.5	-	2.5	-
	A. niger	15.0	70.0	45.0	35.0
	A. ochraceus	12.5	-	-	-
	A. ustus	2.5	-	-	-
	Fusarium moniliforme	15.0	-	75.0	22.5
	Fusarium oxysporium	2.5	-	-	-
	Neurospora sitophila	-	-	-	25.0
	Paecilomyces varioti	-	-	-	2.5
	Cladosporium herbarum	-	-	-	-
	Cephalosporium acremonium	-	2.5	-	-
	Drechslera maydis	7.5	-	50.0	-
	Penicillium verrucosum cyclopium	17.5	22.5	60.0	25.0
	Rhizopus oryzae	10.0	50.0	25.0	17.5
	Mycelia sterilia	-	62.5	-	2.5
	Rhizoctonia solani	-	25.0	-	-
	Curvularia lunata	2.5	-	-	-
2Aβ	*Aspergillus flavus* Link	37.5	65.0	55.0	50.0
	A. fumigatus	-	12.5	-	-
	A. glaucus	-	20.0	-	2.5
	A. niger	12.5	32.5	67.5	35.0
	A. japonicus	2.5	-	-	-
	A. ochraceus	5.0	2.5	-	5.0
	Curvularia lunata	7.5	-	-	-
	Fusarium moniliforme	65.0	25.0	92.5	40.0
	Fusarium oxysporium	22.5	-	-	-
	Drechslera maydis	-	-	15.0	32.5
	Penicillium verrucosum var. *cyclopium*	10.0	22.5	25.0	32.5
	Rhizopus oryzae	82.5	75.0	17.5	25.0
	Rhizoctonia solani	-	-	7.5	-
	Neurospora sitophila	12.5	-	-	-
	Mycelia sterilia	-	25.0	25.0	-

Table 4c.

Location code	Fungi recorded on grains	% occurrence on grains kept in			
		Jute sacs for		Polypropylene sacs for	
		2 months	4 months	2 months	4 months
1Bα	*Aspergillus flavus*	47.5	100.0	50.0	35.0
	A. glaucus	-	2.5	-	5.0
	A. japonicus	-	-	-	5.0
	A. niger	52.5	70.0	82.5	52.5
	A. ochraceus	7.5	-	-	-
	A. ustus	-	-	-	-
	Drechslera maydis	-	-	5.0	62.5
	Fusarium moniliforme	100.0	7.5	75.0	67.5
	Fusarium oxysporium	5.0	32.5	-	7.5
	Penicillium verrucosum var. *cyclopium*	30.0	40.0	50.0	42.5
	Rhizoctonia solani	-	52.5	7.5	-
	Rhizopus oryzae	100.0	92.5	35.0	15.0
	Aspergillus fumigatus	7.5	32.5	-	-
1Bβ	*Aspergillus flavus*	55.0	70.0	57.5	70.0
	A. fumigatus	-	22.5	-	-
	A. japonicus	-	10.0	-	-
	A. ochraceus	7.5	-	-	-
	A. ustus	5.0	-	-	-
	A. niger	42.5	50.0	47.5	27.5
	Cephalosporium acremonium Corda	-	-	5.0	12.5
	Penicillium verrucosum var. *cyclopium*	45.0	17.5	55.0	60.0
	Fusarium moniliforme	42.5	37.5	45.0	75.0
	Fusarium oxysporium	5.0	-	2.5	5.0
	Rhizopus oryzae	40.0	95.0	82.5	75.0
	Aspergillus glaucus	2.5	-	-	-
	Rhizoctonia solani	-	22.5	-	-

Table 4d.

Location code	Fungi recorded on grains	% Occurrence on grains kept in			
		Jute sacs for		Polypropylene sacs for	
		2 months	4 months	2 months	4 months
2Bα	*Aspergillus flavus* Link	45.0	65.0	67.5	50.0
	A. fumigatus	-	2.5	-	-
	A. glaucus	-	-	2.5	7.5
	A. niger	37.5	52.5	15.0	32.5
	A. japonicus	-	2.5	-	2.5
	A. ochraceus	5.0	2.5	5.0	-
	A. ustus	5.0	-	17.5	-
	Drechslera maydis	7.5	-	30.0	17.5
	Curvularia lunata	5.0	5.0	-	-
	Fusarium moniliforme	40.0	5.0	100.0	52.5
	Fusarium oxysporium	2.5	10.0	-	5.0
	Neurospora sitophila	5.0	-	-	-
	Penicillium verrucosum var. *cyclopium*	5.0	5.0	37.5	17.5
	Rhizopus oryzae	30.0	7.5	-	-
	Mycelia sterilia	25.0	-	-	-
	Cladosporium herbarum	2.5	-	-	-
2Bβ	*Aspergillus candidus* Link	2.5	-	2.5	-
	A. flavus	47.5	62.5	47.5	45.0
	A. glaucus	-	-	-	-
	A. niger	37.5	47.5	37.5	50.0
	A. ustus	2.5	-	2.5	-
	A. japonicus	2.5	-	2.5	2.5
	A. ochraceus	-	10.0	-	-
	Cephalosporium acremonium	15.0	-	15.0	-
	Drechslera maydis	-	-	-	27.5
	Penicillium verrucosum var. *cyclopium*	67.5	17.5	67.5	40.0
	Rhizopus oryzae	-	20.0	47.5	37.5
	Rhizoctonia solani	-	2.5	-	-
	Fusarium moniliforme	80.0	27.5	80.0	90.0
	Fusarium nivale	-	10.0	-	-
	Fusarium oxysporium	7.5	5.0	7.5	-
	Fusarium solani	7.5	-	7.5	-

Table 4e.

Location code	Fungi recorded on grains	% Occurrence on grains kept in			
		Jute sacs for		Polypropylene sacs for	
		2 months	4 months	2 months	4 months
3Bα	*Aspergillus flavus* Link	35.0	45.0	40.0	40.0
	A. glaucus	-	5.0	2.5	-
	A. niger	32.5	45.0	72.5	92.5
	A. fumigatus	-	17.5	-	-
	A. ochraceus	-	-	-	-
	Cladosporium herbarum	2.5	-	-	-
	Fusarium moniliforme	20.0	22.5	55.0	95.0
	Fusarium oxysporium	-	-	-	-
	Paecilomyces varioti	32.5	2.5	-	-
	Penicillium verrucosum var. *cyclopium*	20.0	25.0	60.0	40.0
	Rhizopus oryzae	50.0	17.5	12.5	75.0
	Mycelia sterilia	5.0	2.5	-	-
	Rhizoctonia solani	-	-	-	25.0

6.4 Discussion

Decrease in germination is one of the most sensitive indicators of incipient spoilage of stored seeds, including cereal grains. Although ability of grains to germinate may decrease without attendant decrease in processing quality, this reduced viability certainly shows that grain lots are being slowly invaded by storage fungi. There is information in the pertinent literature, on the drastic reduction of germination of grains by storage fungi. For example, Lopez and Christensen (11) stored maize at 19-20% m.c. and 20-25 ^{o}C, some samples free of fungi, others inoculated with *Aspergillus flavus*. After 74 days, the samples free of fungi averaged 97% germination, whilst those inoculated with *A. flavus* averaged 13%. All species of the *A. glaucus* group as well as *A. candidus* and *Penicillium* invade various parts of the seed, including the germ and directly cause or contribute to reduction in germination (12). Present data cannot explain why in some instances fungi recorded on grains kept in jute and woven polypropylene sacs were similar (Table 4a-e) but viability (% germination) of grain differed (Figs 2a and 2b). The better storage sac, woven polypropylene, maintained a lower fungal deterioration of maize grains kept in them; germination of grains was significantly higher (as compared with those kept in jute sacs) after 2-4 months storage (Figs 2a and 2b) and the fungal count increased only slightly (initial 1.2×10^2 - 7.6×10^2 c.f.u.g^{-1}) in 4 months. Presumably, the higher m.c. of grains in jute sacs (14.4-15.6% m.c.) permitted more fungal deterioration action on the germ of the grains resulting in lowering of germination from about 98% to averagely 35% in four months. Corresponding fungal count increased from an initial 1.2×10^2- 7.0×10^4 c.f.u.g^{-1} (two log cycles) over the same period).

Additional work is needed to elucidate the effect of the saprophytic activity of the six most predominant fungi isolated, namely *A. flavus, A. niger, Fusarium moniliforme, F. oxysporium, Penicillium verrucosum* var. *cyclopium* and *Rhizopus oryzae*, on the tensile strength of the two types of storage sacs.

Rice does not seem to have serious storage problems with fungal contamination (Table 3) except that the presence of insects such as *Corcyra cephalonica* and *Ephestia cautella* would require gamma radiation to eliminate the insects. If this is not done, the insects may gnaw the woven polypropylene sacs and cause perforations in them. It has been shown that a dose of 80 Krad will disinfest grains of all insects under the Ghanaian tropic conditions (13).

The use of chemical fumigants for the control of post-harvest losses caused by insects and fungi is well known. The use of methyl bromide, picrin,

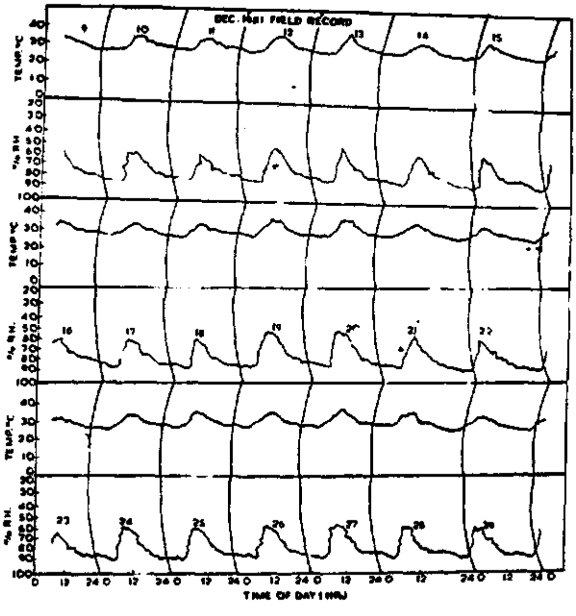

Fig. 7 Record of daily ambient Relative Humidity and Temperature in the Warehouse (Fig.1) during the period 9th-29th December, 1981.

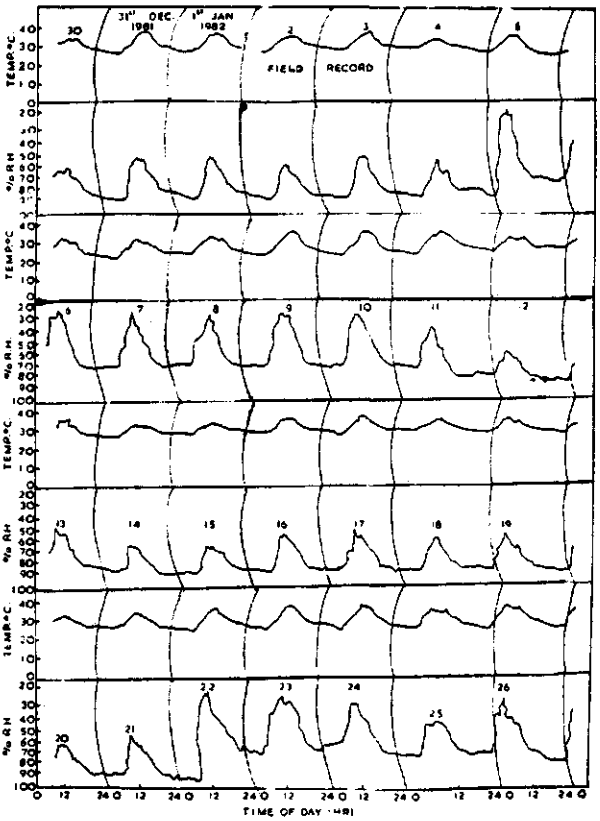

Fig. 8. Graph showing daily record of ambient Relative Humidity and temperature in the Warehouse (Fig. 1) during 30th December, 1981 through 26th January, 1982. (Note the low 25% R.H. recorded on the 22nd January, 1982 during the Harmattan Period.)

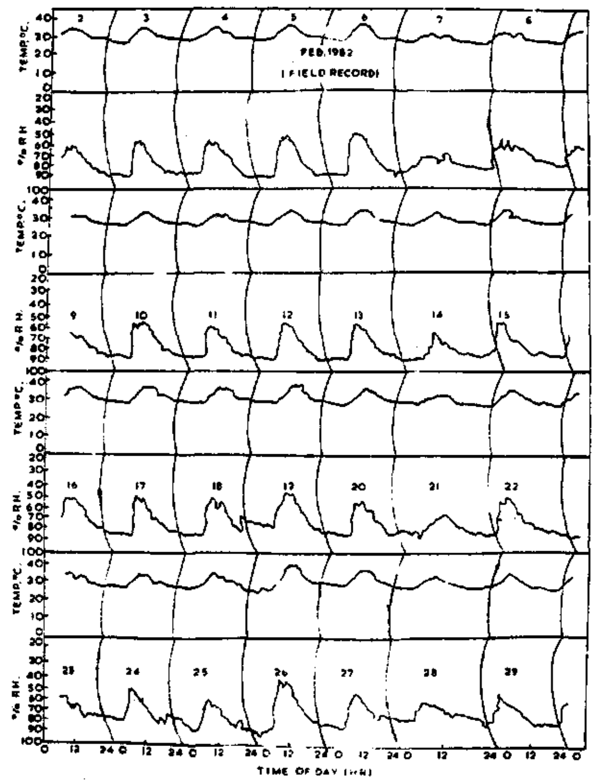

Fig. 9 Graph showing daily record of ambient R.H. and Temperature in the Warehouse (Fig.1) during February 1982.

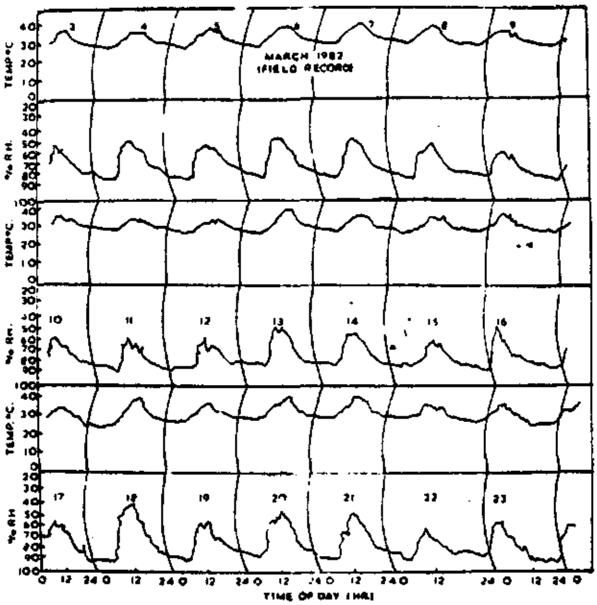

Fig. 10. Graph showing daily record of ambient R.H. and temperature in the Warehouse (Fig. 1) during 3rd - 23rd March, 1982.

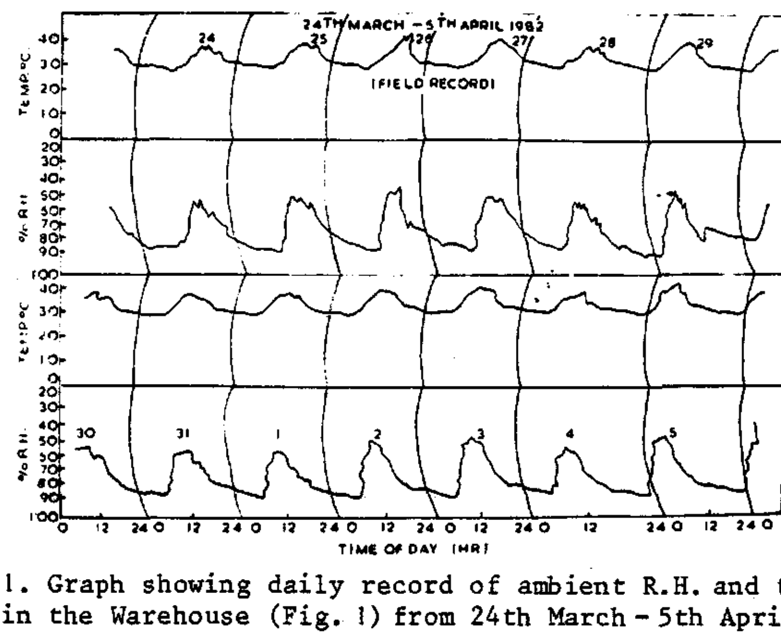

Fig. 11. Graph showing daily record of ambient R.H. and temperature in the Warehouse (Fig. 1) from 24th March - 5th April, 1982.

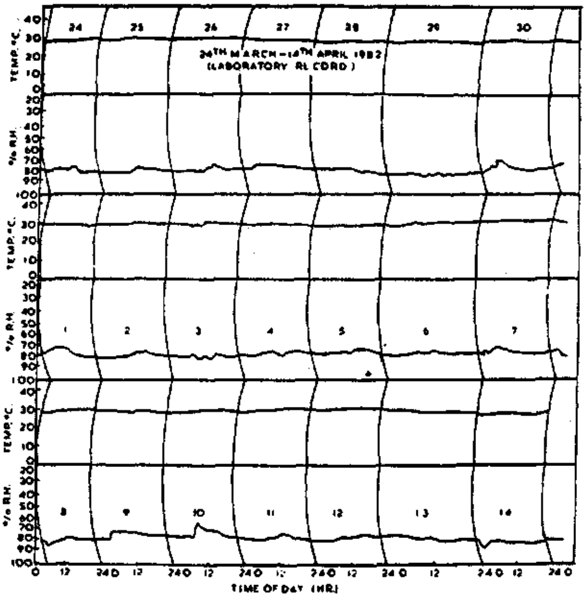

Fig. 12 Graph showing daily record of ambient R.H. and Temperature in the Laboratory from 24th March – 14th April, 1982.
(Note the nearly constant R.H. and Temperature obtained).

ethylene dibromde : methyl bromide (1:1) besides ammonia and sulphur dioxide for control of grain fungi have been reported (14, 15, 16).
For example, ethylene dibromide and chloropicrin killed spores of *A. flavus* and *A. niger* (LD_{95} at 10 mg 1^{-1}) in laboratory test but the same fumigant could not control all fungi if the fungi are inside the grain (17) such as in this investigation. Inhibition of mould growth and formation of mycotoxins has been reported (17, 18, 19) for fumigants methyl bromide, ethylene oxide, sulphur dioxide, chloropicrin and mixtures of ethylene dibromide and methyl bromide. Conversely, grains (wheat) treated with phosphine caused significant increase in yield of aflatoxin B_1 by *A. flavus* NRRL 3251 and a significant decrease of aflatoxin B_1 and G_1 by *A. flavus* NRRL 3145 (20).

In many publications (14-20, etc.) effect of insecticide treatment on some strains of fungi were strain dependent as well as insecticide dependent. In addition, it is established (21) that volatile substances interfere with respiratory mechanisms of spores. The cell wall composition and wall contents vary not only in species of fungi but also the stages such as hyphae conidiophores and spores; mycelia being more susceptible than spores. During this study published here, aerial prophylactic spraying of Warehouse with Actellic 25$^{(R)}$ and Phosphine resulted in the reduction of fungal colony counts and elimination of some fungal species (Figs 3 - 6) but this was followed by an increase in the subsequent sampling week. Additional work is needed to elucidate the effect of Actellic 25$^{(R)}$ and Phosphine gas on the fungi encountered. The continuous use of the insecticides is objectionable because of the residue they leave in our food source. The use of gamma radiation offers a better alternative (22) and storage sac such as woven polypropylene sac is suggested as potential replacement for jute sac in order to prevent reinfection after the terminal irradiation treatment.

6.5 Conclusions

Grains kept in woven polypropylene sacs, under practical field conditions, were significantly (P = 0.01 or 0.05) more viable after 2-4 months storage than those kept in jute sacs. Decrease in germination is an indication of the certainty that grain lots are being slowly invaded by storage fungi resulting in reduced quality. The higher records of fungal population on maize grains kept in jute sacs confirm this.

Evidence is presented to show that prophylactic spraying with insecticides neither kill the fungi on and inside the grains nor in the surrounding air, in

Table 5. Table showing moisture content (%) of maize grains stored in the indicated sacs for sixteen weeks.

Storage sac	Bay 3	Storage period in weeks						
		Initial	2	4	6	8	12	16
Woven poly-pro-pylene	1Aα	11.2±1.3	11.5±0.3	13.0±0.7	12.8±0.3	13.9±0.3	11.6±0.1	13.3±0.2*
	1Aβ		11.6±0.1	13.7±0.6	12.7±0.1	13.3±0.1	12.8±0.2	13.2±0.3
	1Bα		11.7±0.7	13.3±0.2	12.6±0.3	12.8±0.2	11.7±0.3	13.2±0.1
	1Bβ		11.8±0.2	13.2±0.3	12.2±0.3	13.6±0.3	11.5±0.3	13.4±0.3
Jute	1Aα	11.2±1.3	11.6±0.7	14.5±0.3	14.6±0.0	14.9±0.3	15.3±0.7	15.6±0.4*
	1Aβ		11.6±0.4	14.5±0.7	14.0±0.3	14.8±0.3	15.0±0.7	15.8±0.7
	1Bα		11.5±0.6	14.6±0.4	14.8±0.8	14.7±0.5	14.9±1.3	15.9±0.7
	1Bβ		11.8±0.5	14.4±0.3	14.7±0.1	14.9±0.7	15.2±0.0	15.3±1.2

* % Moisture on dry weight basis. Data represent average of four replicates.

the Warehouse. Therefore, other means, notably gamma radiation is required to kill spores of fungi contaminating cereal grains and thus improve the microbiological quality of the grains.

The objective of gamma radiation treatment is to prolong the shelf-life of these grains for a period exceeding one year. The proven ability of gamma radiation to control mould growth will be augmented by the use of woven polypropylene sac as a packaging material for pre- and post-irradiation storage of cereal grains. Woven polypropylene sacs are much lighter in weight than jute sacs and weight for weight, woven polypropylene sacs are significantly stronger than jute sacs (23). Unlike the jute sacs, they are unaffected by water, do not rot in damp conditions, do not absorb moisture, they are clean and do not impart any odour or taint to their contents. A survey conducted by Tripp (4) confirmed that radiation level of 1Mrad (10 KGy) induce only minor changes in the mechanical and chemical properties of packaging woven polypropylene and that no measurable radioactivity is induced by ^{60}Co, ^{137}Cs or by electrons up to 10 MeV, in the normally present elements of the packaging polypropylene. This sac therefore offers many advantages over the traditional jute sac for use in tropical areas like Ghana. It remains to be established which of the two sacs would harbour and support saprophytic activity of fungi and thus acts as springboard for infection of cereal grains kept in it.

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7. Studies on the selection of a suitable packaging material for pre- and post-irradiation storage of cereal grains in Ghana

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Twenty different species of fungi belonging to the genera *Aspergillus*, *Cephalosporium*, *Dreschlera*, *Fusarium*, *Mucor*, *Neurospora*, *Rhizoctonia*, *Rhizopus*, *Penicillium* and *Trichoderma* were isolated from jute and woven polypropylene sacks using the blotter test, solid medium test and the decimal serial dilution technique. There was a highly significant difference (Students' t-test, $P < 0.05$) between the larger number of fungal colonies associated with jute sacks than with woven polypropylene sacks. Correspondingly, more fungal species (16) were isolated from jute sacks than from woven polypropylene sacks (9). The blotter test showed that in the absence of an exogenous supply of nutrients, 88% of the sections of jute sacks supported in vivo growth of fungal spores whilst woven polypropylene sacks could not support the growth of contaminating spores. Evidence is presented that mould and yeast counts on sections of new-woven polypropylene sacks incubated at 80 and 90% relative humidity (R.H.) for four months increased by less than 1 log cycle, whilst in similar sections hung on a line under ambient conditions (75 ± 10% R.H. and 28 ± 3°C) viable counts of spores decreased. Sections of fresh jute sacks similarly treated supported 1 log cycle increase in mould and yeast counts at 80% R.H. and 2 log cycles increase at 90% R.H. after four months of storage. Gamma-ray irradiation (4.0 kGy) reduced the mould and yeast counts on new jute and new woven polypropylene sacks by 1 and 2 log cycles, respectively, but post-irradiation storage at 80% R.H. allowed moulds like *Aspergillus flavus*, *A. niger*, *Fusarium nivale* and *Penicillium verrucosum* var. *cyclopium* to commence growth on jute sacks. This presumably, may act as a springboard for infecting grain contents of jute sacks. Inert woven polypropylene sacks failed to support fungal growth.

Key words: Packaging materials; Cereal grains; Mycoflora; Jute and woven polypropylene sacks; Gamma-ray irradiation

Introduction

Flatman (1977) suggested that jute sacks should be replaced by synthetic sacks such as those woven from polypropylene for storage of cereal grains, grain products, cocoa, animal feed etc. in the tropics. However, warehousing agents and the Ghana Cocoa Marketing board continue to use jute sacks because they are cheaper. The existing standard practice in Ghana of bagging cocoa, rice, maize, sorghum, groundnuts etc. in jute sacks causes defects and loss of over 30% of the annual harvest due to insects and fungi. Recent 1983 estimates by the Ministry of Agriculture showed that stored maize worth US $2 million was lost due to insect and fungal activity. A search for alternative packaging materials for cereal grains and grain products is desirable if maximum benefits are to be derived from radiation decontamination of mould on cereal grains and grain products.

Amoako-Atta (1979) showed that radiation insect disinfestation of cocoa and other seed grains using 0.8 kGy was feasible when kept in woven polypropylene sacks. But this dose was insufficient for killing fungi. Odamtten et al. (1980a) reported that gamma irradiation (4.0 kGy) in combination with heating (60°C for 30 min) administered under high ambient humidity conditions (> 85% R.H.) was effective in preventing both growth and aflatoxin B_1 formation by *Aspergillus flavus* NRRL 5906 in maize stored in woven polypropylene sacks for 4 months at 80% R.H. and 28°C. This same combination treatment decreased aflatoxin B_1 formation and eliminated Enterobacteriaceae in animal feed and cotton seeds (Odamtten et al., 1980b). There is no information in the literature on the comparison of the efficacy of jute and woven polypropylene sacks in curtailing the re-contamination of grains by fungi. Since maize grains and other cereals are stored over long periods, as buffer stocks before use, it is important to evaluate the performance of the two packaging materials microbiologically because any possible benefit from radiation decontamination of cereal grains would be negated if the packaging material should support 'quiescent' growth of residual fungal spores after radiation treatment, possibly allowing influx of spores through its lattice work to re-contaminate the contents. Hueck (1965) stated that the mechanical penetration of fungi into a storage sack could be an initiating mechanism for chemical and mechanical damage and soiling. Such mechanical penetration would eventually manifest itself in the reduction of the intrinsic tensile strength of the packaging material, lowering the ability to withstand rough transit and warehouse handling.

Our objectives in this paper are firstly to survey the natural mycoflora associated with jute and woven polypropylene sacks and find out the effect of gamma irradiation on the moulds. Secondly, the evaluation of pre- and post-irradiation microbiological quality of the sacks and contents after extended storage under ambient warehouse conditions (75 ± 10% R.H. and 28 ± 3°C) and also representative storage conditions similar to tropical high humidity conditions, (80 and 90% R.H.) conducive for growth of moulds.

Materials and Methods

Mycoflora of jute and woven polypropylene sacks

Three different methods were used; a solid medium test, a blotter test and a serial dilution technique.

Solid medium test

Sections (5 × 5 cm) of either fresh jute or woven polypropylene were placed in sterile petri dishes (9.0 cm diameter) containing Sabouraud's maltose agar. Each of the fourteen replicate petri dishes contained only one section. The plates were incubated at 28 ± 3°C for 5–7 days. The species of fungi appearing after 7 days, the percentage of each fungus and the total number of fungal colonies appearing were recorded. The experiment was repeated four times.

Blotter test

In the blotter test, the sections (5 × 5 cm) of either fresh jute or woven polypropylene sacks were placed, one in each of fourteen sterile 9.0 cm diameter petri dishes each containing Whatman's filter paper moistened with sterile distilled water without any further additional treatment. The dishes were then incubated for up to 14 days at 28 ± 3°C (Gemawat, 1968). The experiment was repeated four times and the percentage number of sections showing visible fungal infection viewed under a Prior binocular microscope (W.R. Prior & Co. Ltd., Herts, England) were noted.

Serial dilution technique

In the third series of tests, twelve sections of fresh or used sacks of jute and woven polypropylene (4 × 18 cm) were enclosed in desiccators (with inner environment) adjusted to relative humidities of 80 or 90% with potassium hydroxide solutions after the method of Solomon (1952). There were thus four dessicators with ambient R.H. 80% each containing either 12 sections of fresh jute, fresh woven polypropylene, used jute or used woven polypropylene sacks. In a parallel experiment, four dessicators at 90% R.H. contained the same series of cut sections as mentioned above. Similar sections (4 × 18 cm) of both fresh and used woven polypropylene sacks were hung on a line outside the laboratory exposed to ambient environmental conditions (75 ± 10% R.H. and 28 ± 3°C) for 4 months. We exposed 12 sections of each packaging sack in the fresh or used state. The initial and final mould and yeast counts of packaging materials were determined by placing samples into 100 ml 0.1% Peptone as diluent in 250 ml Erlenmeyer flasks shaken in a Gallenkamp orbital shaker at 140 rev./min for 30 min. From this stock suspension, the serial decimal dilution technique was employed and the spores were raised on oxytetracycline glucose yeast extract agar (OGYE, Oxoid CM 545) for 3 days at 28°C. The number of colonies appearing were counted and the number of cfu/72 cm^2 (area of strips) was calculated.

Effect of gamma irradiation on the natural mycoflora

Cut sections (4 × 18 cm) of both used and fresh jute and woven polypropylene sacks were irradiated with 0.0, 3.0 and 4.0 kGy of gamma radiation from a ^{60}Co source (Gamma Cell 220, Atomic Energy of Canada Limited) with a dose rate of 2.13 kGy/h. There were 10 cut sections per dose for each type of sack in the used and fresh state. The mould and yeast counts/72 cm^2 of the sections were determined in the same manner as above. Concurrently, 12 jute and 12 woven polypropylene sacks were each filled with 50 kg of maize and stored in the warehouse of the Grains

Warehousing Company, Tema. We compared the fungal population of the grains in the two types of packaging materials initially and after 4 months storage.

Results

Results of the survey of the mycoflora of jute and woven polypropylene are presented in Fig. 1 and Table I. Twenty different fungi were isolated in the solid medium test (Fig. 1). There was a highly significant (Student's t-test, $P < 0.05$) difference between the higher number of fungal colonies associated with the incubated jute sack sections and the lower numbers counted on the sections of woven polypropylene sacks. Correspondingly, more fungal species were recorded on jute than on woven polypropylene sacks (Table I). Results of the blotter test showed that in the absence of an exogenous supply of nutrients, 88% of the section of jute sack supported growth of colonies of *Aspergillus flavus, A. niger, A. japonicus, Fusarium*

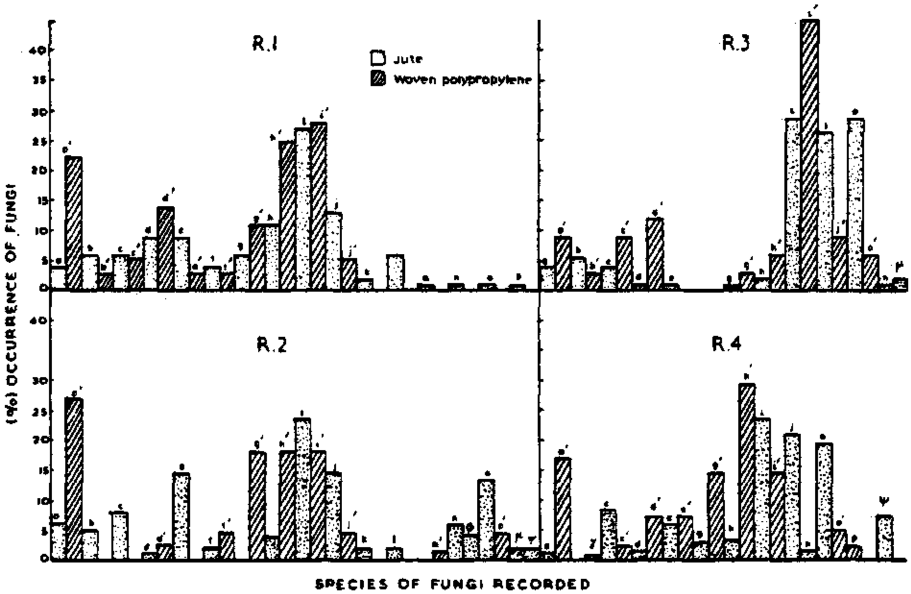

Fig. 1. Histograms showing association of fungi with cut sections of jute, and woven polypropylene sacks, placed on Sabouraud's maltose agar for 7 days at 28 ± 3°C. R.1–R.4 are replicate experiments. Code:

a, *Aspergillus niger*	h, *Mucor pusillus*	o, *Mycelia sterilia*
b, *Aspergillus flavus*	i, *Rhizopus oryzae*	p, *Fusarium semitectum*
c, *Drechslera maydis*	j, *Rhizoctonia solani*	γ, *Cephalosporium acremonium*
d, *Fusarium moniliforme*	k, *Fusarium spp.*	μ, *Penicillium digitatum*
e, *Fusarium oxysporium*	l, *Aspergillus parasiticus*	ψ, *Penicillium verrucosum* var. *cyclopium*
f, *Fusarium nivale*	m, *Aspergillus japonicus*	
g, *Neurospora sitophila*	n, *Aspergillus ochraceus*	φ, *Penicillium expansum*.

TABLE I

Number of fungal species and total number of colonies recorded on the indicated sacks placed on Sabouraud's maltose agar at 28 ± 3°C for 5 days

Replicates	Number of fungal species recorded		Total number of fungal colonies recorded on	
	WP [a]	J [b]	WP	J
R_1	10	16	42	100
R_2	9	16	44	100
R_3	9	14	33	95
R_4	9	12	40	115

[a] Woven polypropylene sack.
[b] Jute sack.

moniliforme, *Penicillium verrucosum* var. *cyclopium* and *Curvularia lunata* which could be identified after 21 days of incubation. No fungal growth however, was recorded on the woven polypropylene sections in the blotter test. This material, therefore, could not support de novo growth of fungal spores.

There was a slight change (less than 1 log cycle) in the mould and yeast counts on sections of fresh woven polypropylene sacks incubated at 80 and 90% R.H. for 4 months whilst fungal spores on used woven polypropylene sacks hung outside the laboratory decreased after 4 months of incubation (Table II). Fresh jute supported 1 log cycle increase in viable counts of mould and yeasts at 80% R.H. and about 1.3 log cycle increase at 90% R.H. after 4 months.

Both fresh and used jute and woven polypropylene sacks carried appreciable amounts of viable fungal spores (Table III), which were reduced by about 2–3 log cycles by 4.0 kGy of gamma radiation. Although irradiation lowered the microbial burden on the two types of sacks, spores of *A. flavus*, *A. niger*, *Fusarium nivale* and *Penicillium verrucosum* var. *cyclopium* remained viable after exposure to 4.0 kGy of gamma irradiation (Fig. 2).

TABLE II

Mould and yeast counts on sections of jute and woven polypropylene sacks kept under the indicated humidity conditions for four months at 28 ± 3°C

Storage humidity (% R.H.)	Mould and yeast counts ($\log_{10}$ cfu/72 cm^2) on:							
	Fresh WP [a]		Used WP		Fresh J [b]		Used J	
	Initial	4 mnth.	Initial	4 mnth.	Initial	4 mnth.	Init.	4 mnth.
80	1.00	1.30	3.73	2.0	2.8	3.8	5.6	5.9
90	1.00	1.80	3.73	2.1	2.8	4.1	5.6	5.4
Open air (75 ± 10% R.H.)	1.00	0.60	3.73	2.9	3.8	2.7	5.6	5.6

[a] Woven polypropylene sacks.
[b] Jute sack.

TABLE III

The effect of gamma irradiation on the fungal contamination of fresh (new) and used packaging materials

Type of packaging material	Mould and yeast count ($cfu/72\ cm^2$) on fresh sacks		Mould and yeast count on used sacks		($cfu/72\ cm^2$) [a]	
	0.0 kGy	3.0 kGy	4.0 kGy	0.0 kGy	3.0 kGy	4.0 kGy
Jute	1.5×10^4	1.3×10^3	9.3×10^2	9.2×10^5	5.9×10^3	4.3×10^2
Woven polypropylene	1.1×10^3	9.3×10^2	7.3	1.5×10^3	6.7×10^2	6.0×10^1

Data represents the mean of four replicates.

[a] Sacks that had been used for storing maize for 4 months were used for assessing the effect of irradiation on contaminating moulds.

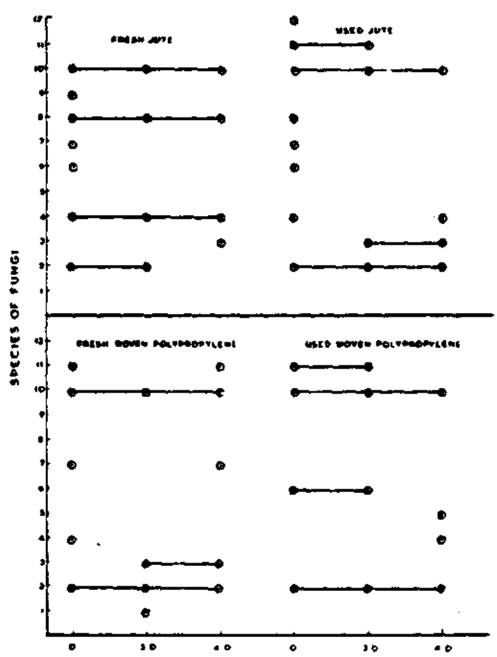

Fig. 2. Scatter graph showing absence or presence of fungi on non-irradiated (0 kGy) and irradiated (3.0 and 4.0 kGy) fresh and used storage sacks. Code:

1. *Aspergillus candidus*
2. *Aspergillus flavus*
3. *Aspergillus glaucus*
4. *Aspergillus niger*
5. *Aspergillus ustus*
6. *Curvularia lunata*
7. *Fusarium moniliforme*
8. *Fusarium nivale*
9. *Fusarium oxysporum*
10. *Penicillium verrucosum var. cyclopium*
11. *Rhizopus oryzae*
12. *Trichoderma viride*.

TABLE IV

Mould and yeast counts of maize grains stored in the fresh sacks for 4 months in the Grains Warehousing Company's Warehouse at Tema

Type of packaging material	Initial mould and yeast count on OGYE (cfu/g)	Final mould and yeast count on OGYE (cfu/g)
Jute	1.2×10^2	7.6×10^4
Woven polypropylene	1.2×10^2	6.8×10^2

Data represents the mean of six readings.

During the four months storage of maize grains in fresh jute sacks, the mould and yeast counts increased by more than two log cycles (from 1.2×10^2 to 7.6×10^4 cfu/g). On the other hand, there was only a slight increase (1.2×10^2 to 6.8×10^2 cfu/g) in the mould and yeast counts on maize grains stored in woven polypropylene sacks for the same period in the warehouse.

Discussion

This paper reports the first record of fungi associated with jute and woven polypropylene in Ghana (Fig. 1) and thus provides information which warrants further research on the effect of the mycoflora on the decomposition rate of jute fabric during storage under local warehouse conditions.

Fungi and insects alike continue to take a heavy toll of our stored cereal grains due to the persistent use of jute sacks by our warehousing agents. Jute sacks contain sufficient nutrients to support fungal growth on 88% of the sections placed on sterile filter paper. This implies that fungi can attack this packaging material and cause mechanical and chemical damage and soiling as stated by Hueck (1965).

Gamma irradiation (4.0 kGy) could reduce the viable mould and yeast counts on both jute and woven polypropylene sacks by 2–3 log cycles (Table III) but subsequent storage of jute sacks at high ambient relative humidity (> 80% R.H.) resulted in resumed growth (Table II) of fungi such as *A. flavus, A. niger, F. nivale, P. verrucosum* var *cyclopium* and *Curvularia lunata*. The presence of fungal spores on the fabric of jute sacks not only causes soiling but also alters the appearance of the sacks by their coloured metabolites. Secondly, spores may presumably pass through the lattice work of the sack and act as a spring-board for contaminating grain contents. This partly explains why viable mould and yeast counts of maize grains stored in jute sacks increase by 2.8 log cycles (from an initial 1.2×10^2 to 7.6×10^4 cfu/g) in 4 months.

On the other hand, woven polypropylene sacks did not support fungal growth. Maize grain contents were of better microbiological quality (6.8×10^2 cfu/g) than those kept in jute sacks for 4 months. According to Beuchat (1978) fungi often decrease germination capacity and cause discolouration of grains and seeds. Using the criterion of viability (percentage germination) as an index of fungal deterioration of maize grains, Odamtten (1982) demonstrated that maize grains stored in woven polypropylene sacks under field conditions were significantly ($P < 0.05$) more viable than grains stored in jute sacks after 4 months storage period. Based on the microbiological evidence that jute sacks support fungal growth, complementary studies are in progress to (a) confirm that at sufficiently high tropical ambient humidities (> 80% R.H.) fungi contaminating jute packaging sacks could reduce drastically the intrinsic tensile strength of the sacks due to their chemical activity (b) single out fungi that are only surface contaminants of sacks and have no cellulolytic activity. Spores of such fungi can cause soiling and presumably pass through lattice work of sack and subsequently contaminate sack contents. Findings of these studies will be reported in a subsequent paper.

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8. Mycological and physical parameters for selecting suitable packaging material for pre- and post-irradiation storage of cereal grains in Ghana

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Owing to the rough warehouse handling of storage sacks in tropical areas in Africa, a suitable storage sack should not support de novo growth of fungal spores because this would reduce the tensile strength of the packaging material and act as a springboard for infecting grain contents. This paper reports the effect of activity of saprophytic fungi on the tensile strength of jute and woven polypropylene sacks. New woven polypropylene sacks carried lower levels of fungal spores (1.3×10^1 cfu/72 cm^2) than jute sacks (3.0×10^3 cfu/72 cm^2). The natural mould penetration and growth was examined on sections (4×5 cm) of both jute and woven polypropylene after previous incubation at relative humidities of 55, 60, 65, 70, 75, 80, 90 and 95% for 10 weeks by placing them on Sabouraud's Agar. There was a significant difference ($P = 0.05$ level of significance) between the higher penetration of mould growth on jute sacks and that obtained on woven polypropylene sacks. Saprophytic fungi (*Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. japonicus, A. parasiticus, A. ustus, Fusarium oxysporium, F. moniliforme, Penicillium verucosum* var. *cyclopium, Rhizopus oryzae* and *Trichoderma viride*) isolated from jute sacks reduced tensile strength, measured by an Instron Model 1026, by 50–75% after 10 weeks at 90% R.H. Same fungal species on woven polypropylene sacks did not alter the tensile strength. Woven polypropylene sacks did not absorb moisture whilst the moisture content of jute sacks increased by 5.3–6.0% in 10 weeks at 90% R.H. with concomitant increase in mould and yeast counts by 1–2 log cycles. Evidence is presented to show that there was a positive correlation between the final mycoflora on jute sacks and loss in tensile strength. No correlation, however, was found between the total aerobic bacteria on jute sacks and the concomitant reduction in tensile strength. Fungi therefore play a major role in the reduction of tensile strength of jute sacks. Sterilization by gamma irradiation (8.0 kGy) of jute and woven polypropylene sacks did not affect their intrinsic tensile strength. Woven polypropylene sacks therefore have many microbiological and physical advantages over the traditional jute sacks to merit their use for grain storage in tropical areas like Ghana.

Key words: Fungi; Jute sacks, tensile strength; Polypropylene sacks, woven; Cereal grains

Introduction

The sanitary requirements for preventing food infection and poisoning are embodied in food legislation throughout the world. Selection of the most suitable packaging material can ultimately result in improved shelf-life and better quality while reducing costs, particularly by avoiding undue food losses and waste (Elias, 1979). Packaging research has therefore aimed at developing flexible, light-weight containers capable of withstanding rough handling and storage while retaining their protective properties (Elias, 1979; Killoran and Wierbicki, 1966).

The measurement of extent of fungal growth and extent of deterioration of food including cereal grains and grain products is promising and experimentally useful (Tuite, 1978). In this regard, fungal activity on grains contributing to post-harvest losses and quality deterioration has been well documented (Christensen, 1957; Christensen and Kaufmann, 1969; Odamtten, 1981; Oyeniran 1973a, b; Quasem and Christensen, 1958, 1960; Wallace and Sinha, 1962). But there are limited studies on the effect of contaminating moulds on storage sacks. The tropical environment is amply laden with spores of microorganisms which require the availability of appropriate substrate, humidity, temperature and pH to commence growth. Ayerst, (1969) stated that some fungi are indeed able to make mycelial growth at relative humidities (R.H.) well below that of other groups of microorganisms. In addition, fungal hyphae can penetrate into a substrate, exploiting substrate by their ability to translocate nutrients. According to Eggins and Allsopp (1975) such growth of fungi into substrates often enables growth and breakdown to continue even when adverse humidities may prevail at the surface. Hueck (1965) stated that the mechanical penetration of fungi on a storage sack could be an initiating mechanism for chemical damage because fungi can attack materials and cause mechanical and chemical damage and soiling.

Chemical damage is caused by all four major groups of fungi. The presence of fungal spores and mycelium on cellulose material, plant fibers such as cotton (*Gossypium hirsutum* L.), hemp (*Cannabis sativa*), sisal (*Agave* spp.) textiles and jute (*Corchorus capsularis, C. olitorius*) is objectionable because of the soiling of material caused by their coloured spores and metabolites.

Secondly, several fungi are able to cause damage to materials due to their ability to produce extracellular cellulases. Chemical assimilatory damage by fungi mainly manifests itself, in economic terms, as either weight loss (as in food products) or by loss in tensile strength of sack or fabric.

A good packaging material should not harbour and support growth of contaminating fungi. It is important, therefore, to evaluate the packaging material microbiologically under adverse and favourable conditions that would promote growth after exposure of the material to gamma radiation. Bothast et al. (1979) reported that fungal growth during transit shipment of corn soya milk in conventional multiwall paper bags was confined to the bag surface and was not severe. However, when the conventional bags were subjected to conditions conducive to growth, mould penetrated all layers. In contrast, polyethylene coated bags were more resistant to moisture and fungal penetration unless the polyethylene coat was fractured. Once penetrated, the polyethylene-coated bags were no better than the conventional ones. We aimed at measuring changes in tensile strength and microbiological quality of jute and woven polypropylene sacks exposed to environmental conditions conducive to mould growth. Our paper provides the first microbiological and physical information which would assist in the eventual selection of the more suitable of the two types of sacks for packaging of cereal grains in humid tropics after irradiation treatment.

Materials and Methods

New and unused jute sacks were obtained directly from the Grains Warehousing Company, Tema, Ghana. Jute sacks are presently the conventional packaging material for storing of 50 kg weight of bulk maize in Ghana. Woven polypropylene fabrics (40 warps × 35 tex, 100 tex: the lattice pattern) were purchased from Polyproducts® Factory, Ring Road Industrial Area, Accra.

Qualitative estimation of the influence of storage relative humidity on the development of the natural mycoflora in sacks

To examine the influence of relative humidity on the development of mycoflora on the sacks, ten sections (4 × 18 cm) of either jute or woven polypropylene sacks were stored in dessicators with inside R.H. adjusted to 55, 60, 70, 75, 80, 85, 90 or 95% by the method of Solomon (1952). The set up was kept at 30 ± 2°C for 10 weeks. Thereafter, approximately, 4 × 5 cm sections of either jute or woven polypropylene sacks stored at the preselected R.H. values were aseptically cut and placed on Sabouraud's Maltose Agar in 9.0 cm diameter Petri dishes. There were twenty replicates for each storage humidity and each plate carried only one cut section of the sack. After 3 days incubation at 30 ± 2°C, a subjective evaluation was made to estimate the extent of fungal penetration. A modification of the method of Bothast et al. (1979) was employed. A value of 0 was assigned to sections yielding no fungal growth, 1 to sections one-quarter covered with fungi, 2 to sections half covered, 3 to sections three-quarters covered with fungi and 4 to sections completely covered.

Quantitative assessment of microbiological load on jute and woven polypropylene sacks

Approximately 72 cm^2 of the remaining sections of jute and woven polypropylene sacks kept at R.H. conditions of 55–95% and 30 ± 2°C for 10 weeks were used in assessing quantitatively, the microbiological load of the sacks. The sections were aseptically transferred into 250 ml Erlenmeyer flasks containing 100 ml of 0.1% Peptone diluent. Each flask was shaken at 140 rpm for 30 min in a Gallenkamp Orbital shaker, and serial dilutions were plated on either Oxytetracycline glucose yeast extract agar (OGYE), (Oxoid CM 545) for mould and yeast count or plate count agar (PCA) (Oxoid CM 325) for assessment of the total number of aerobic bacteria. All plates were incubated at 30 ± 2°C and colonies were counted after 3 days of incubation. The predominant moulds were identified based on cultural and morphological characteristics.

Effect of the natural mycoflora on tensile strength of packaging materials

To evaluate the effect of the natural mycoflora on the tensile strength of both packaging materials kept under conditions conducive to fungal growth, two equal sections (4 × 18 cm) of either jute or woven polypropylene were hung on a hook held by cotton wool plug inside one liter Erlenmeyer flasks.

About 250 ml potassium hydroxide solution (11.8% w/w) provided an ambient R.H. of 90% (Solomon, 1952). Six Erlenmeyer flasks containing either jute or woven polypropylene were incubated at 30 ± 2°C for 10 weeks. Samples which served as control were sterilized with 8.0 kGy of gamma radiation from a ^{60}Co source (Gamma Cell 220, Atomic Energy of Canada Limited). Thereafter approximately 72 cm^2 of the sections were assessed microbiologically in the same manner as described above.

We measured the extent of mould deterioration (if any) of the two packaging sacks using the criterion of percentage change (loss) in tensile strength measured on an Instron Universal Testing Instrument, Table Model 1026 (Instron Limited, High Wycombe, Bucks, U.K.). Sections of the jute and woven polypropylene sacks (4 × 18 cm) were held taut in the sample-holding column of the crosshead. The samples were given a full-scale load range of 0–50 kg on a scale factor 5. Record of controls were superimposed on the deteriorating samples to enable us to measure the difference in tensile strength directly. The calculation of the percent loss in tensile strength was obtained from the mathematical relationship:

$$y = (1 - b/a) \times 100$$

Where a is the peak extension for the applied load on control sample, b the peak extension for the applied load on test sample and y the percent loss in tensile strength.

We assumed initially that all fungi appearing after the incubation period contributed to the decrease in tensile strength. As a follow up, we studied the role of selected individual fungal species in decreasing the tensile strength of jute sack.

Sections of jute sack (4 × 18 cm) were first sterilized by 8.0 kGy of gamma radiation, and the sections (six replicates for each fungus) were inoculated by smearing section using a micropipette containing 1.0 ml aliquot of 2% Tween-80 solution suspending 7.2×10^5 spores ml^{-1} of either *Aspergillus flavus*, *A. niger*, *A. parasiticus* or *Rhizopus oryzae*. A Hawksley Haemacytometer Cristalite B.S. 748 (Hawksley and Sons Limited, Sussex, U.K.) was used in estimating the spore density. The inoculated sections were then incubated in 1 l Erlenmeyer flasks on hooks hanging from the cotton wool plugs with an ambient R.H. of 90% provided by potassium hydroxide solution (Solomon, 1952). The change in tensile strength after 10 weeks incubation at 30 ± 2°C was estimated as above.

Moisture content

This was determined in duplicate. Jute sack samples (10 g) were dried at 103°C for 16 h and then reweighed after cooling in a desiccator. Results are expressed on wet weight basis.

Results

Less than half (mean score ≤ 2) of the sections of woven polypropylene sacks were covered with moulds when they were placed on Sabouraud's maltose agar after 10 weeks incubation at R.H. 55–75% and the moulds never covered three quarters (mean score = 3) even at storage humidity 80–95% R.H. (Fig. 1). In contrast to this, more than half of the sections of the jute sack were covered with sporulating fungi when placed on the same medium after 10 weeks storage at 60% R.H. and this growth increased to total coverage (mean score = 4) of sections at R.H. 90 and 95%, respectively. Statistical analysis (t test) showed that differences observed in the extent of fungal penetration between jute sack sections and woven polypropylene sections at each storage humidity were significant ($P < 0.05$).

The quantitative assessment of microbiological load measured in cfu/72 cm^2 of surface using the dilution plate method confirmed the trend we obtained employing the hedonic infection scale (Fig. 1). There were more fungal species associated with

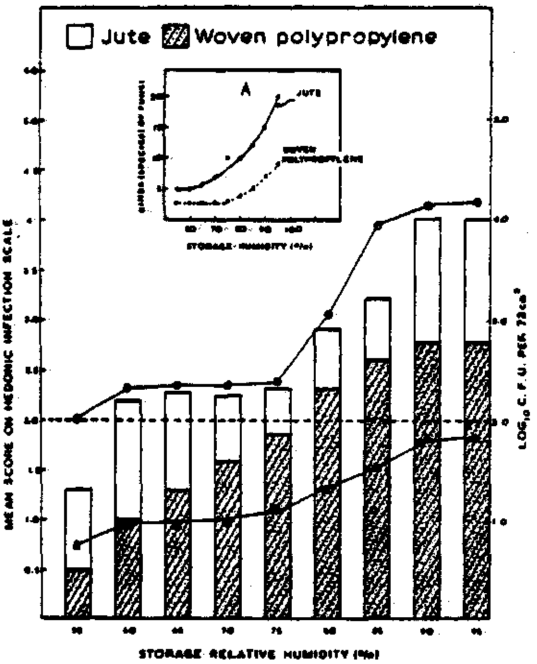

Fig. 1. Qualitative and quantitative estimation of the influence of storage relative humidity (R.H) on the extent of fungal development and penetration into sections of jute and woven polypropylene sacks incubated at 55–95% R.H. for 10 weeks at 30 ± 2°C. Qualitative by infection hedonic scale (1–4). Quantitative by dilution plate method ($\log_{10}$ cfu/72 cm^2): O——O, jute; ▲——▲, woven polypropylene; - - - - - -, line indicating section half-covered with fungal growth. Inset A: Relationship between number of species isolated from indicated sack material and the storage humidity.

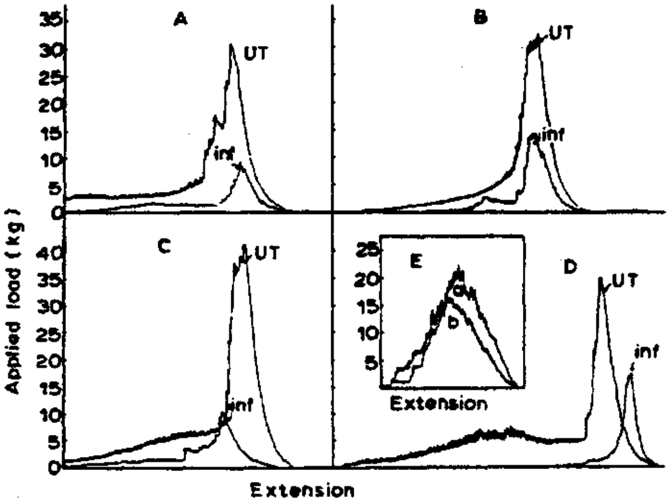

Fig. 2. Chart records of tensile strength characteristics of control (UT) and infected (Inf) samples of jute during measurement on an Instron Table Model 1026. A–D are replicates. Inset E is an example of same measurement with woven polypropylene. Crosshead speed = 100 mm/min; chart speed = 50 mm/min; Load range 0–50 kg (full scale); scale factor = 5.

jute sacks than with woven polypropylene sacks at each storage humidity investigated (Fig. 1A inset).

Saprophytic fungi isolated from woven polypropylene did not reduce significantly the tensile strength of the sections of woven polypropylene (Fig. 2E inset). Woven polypropylene sack did not absorb moisture. A loss in tensile strength of 50–75% caused by the natural mycoflora on jute sacks namely *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. japonicus*, *A. parasiticus*, *A. ustus*, *Fusarium moniliforme*, *F. oxysporium*, *Rhizopus oryzae* and *Trichoderma viride* was recorded. The sections of jute sacks absorbed moisture (from initial 11.4–11.6% to final 17.1–17.6%) with a concomitant increase in mould and yeast count by about 1–2 log cycles in 10 weeks.

There was a positive correlation between the final fungal population on jute sack and the extent of reduction in tensile strength jute sack (Fig. 3a) whilst no correlation was found between the total aerobic bacteria count and the percentage reduction in tensile strength (Fig. 3b). Test of significance of the regression coefficient showed only the effect of fungi to be significant ($P < 0.05$).

Gamma irradiation (8.0 kGy) did not change significantly the intrinsic tensile strength of jute and woven polypropylene sacks. However, inoculation of the jute sack sections with 7.2×10^5 spores ml^{-1} of selected individual fungi namely *A. flavus*, *A. niger*, *A. parasiticus* and *R. oryzae* followed by incubation at 90% R.H. for 10 weeks resulted in a comparatively smaller percentage decrease (10.5–17.1%) in tensile strength in the order *A. flavus* > *R. oryzae* > *A. parasiticus* > *A. niger* (Table 1).

TABLE I

Reduction in tensile strength of jute inoculated with the same initial number of spores of indicated fungi and incubated at 90% R.H. and $30 \pm 2°C$ for 10 weeks

Fungal species	Replicate	%Moisture content		Inoculum size (ml)		% Loss in tensile strength (mean ± S.E. [b]
		Initial	Final	Initial	Final	
Aspergillus	1	11.6 ± 0.3	17.8 ± 0.2			
flavus	2		18.6 ± 0.3			
	3		17.6 ± 1.2	7.2×10^5	3.8×10^7	17.1 ± 3.1
	4		18.2 ± 0.7	(5.9) [a]	(7.6)	
	5		16.8 ± 0.8			
	6		17.4 ± 0.5			
Aspergillus	1	11.6 ± 0.3	17.5 ± 0.7			
niger	2		18.1 ± 0.3			
	3		17.3 ± 0.4	7.2×10^5	4.2×10^7	10.5 ± 6.0
	4		17.1 ± 0.3	(5.9)	(7.6)	
	5		18.8 ± 0.1			
	6		18.2 ± 0.2			
Aspergillus	1	11.6 ± 0.3	17.5 ± 0.3			
parasiticus	2		17.7 ± 0.7			
	3		17.8 ± 0.4	7.2×10^5	3.3×10^7	11.3 ± 5.4
	4		17.6 ± 0.2	(5.9)	(7.5)	
	5		17.6 ± 0.3			
	6		18.1 ± 0.1			
Rhizopus	1	11.6 ± 0.3	18.2 ± 0.7			
oryzae	2		17.6 ± 0.3			
	3		17.7 ± 0.2	7.2×10^5	5.8×10^7	12.7 ± 8.2
	4		17.3 ± 0.5	(5.9)	(7.8)	
	5		17.1 ± 0.1			
			18.1 ± 0.1			

[a] Log_{10} cfu/ml are in parentheses.
[b] Value represents mean of six replicates.

Discussion

In the absence of an exogenous supply of nutrients, jute sacks could support a de novo growth of contaminating mycoflora on 88% of the cut sections whilst no fungal growth occured on the woven polypropylene sections under the same conditions (Odamtten and Kampelmacher 1985). The growth of fungi on the sections of jute and woven polypropylene placed on Sabouraud's maltose agar is not unique but the striking difference is the ability of the moulds to grow well even after being at adverse relative humidity of 55% for 10 weeks. Eggins and Allsopp (1975) stated that such quiescent growth of fungi into substrates enables growth and breakdown to continue even under adverse prevailing humidities at the surface. The significant difference ($P < 0.05$) observed in the greater extent of fungal contamination and penetration of jute than of woven polypropylene sections for each storage humidity (55–95% R.H.) confirms our previous findings (Odamtten and Kampelmacher, 1985). Siu (1951) summarised research findings on cellulolytic activity of fungi and he placed *Aspergillus fumigatus* and *Trichoderma viride* among the strongly cellulolytic group, the moderately cellulolytic ones were *Fusarium moniliforme, F. oxysporium* and *F. solani*. The moulds *Aspergillus japonicus* and *A. ochraceus* were placed in the weak cellulolytic category of fungi whilst *A. candidus, A. flavus* and *R. oryzae* were classified as non-cellulolytic. Basu (1948) reported that his strain of *A. niger* on jute fibre was non-cellulolytic and *A. ustus, A. terreus* and *A. fumigatus* were cellulolytic. Reese and Downing (1951) classified twelve *Aspergillus* spp. according to their cellulolytic ability and he placed *A. flavus, A. ochraceus* and *A. niger*, also isolated in the present study, in the non-cellulolytic group. On the other hand, Flannigan (1970) reported *A. fumigatus* and *A. niger* isolated from barley kernels as cellulose decomposing and Mazen (1973) found *A. flavus* isolated from soil of low cellulolytic activity. Presumably, cellulolytic activity of fungi might be species and strain specific as well as depending on the type of substrate which is being metabolised by the fungus. Several members of the genus *Aspergillus* appear to be capable of hydrolysing the β-(1-4)-glucosidic linkage in the cellulose chain (Alexander, 1961; Raper and Fennel, 1972; Mazen, 1973; Reese and Downing, 1951; Steward and Walsh, 1972). But this does not itself determine whether they can attack cellulose or not since many non-cellulolytic microorganisms possess the C_x enzyme carrying out that reaction (Reese et al., 1950).

Judging from our findings, we surmise that the decrease in tensile strength of 50–75% we obtained after 10 weeks in jute sacks could be attributed to the possible cellulolytic activity of *A. fumigatus, A. ustus, Fusarium oxysporium* and *T. viride*. In vitro cultural studies are presently in progress to confirm the cellulolytic potential of these fungi isolated from the jute sack.

Reese and Downing (1951) considered as inactive those organisms which effect a loss in tensile strength of fabrics of less than 15% in two weeks. Our findings on the reduction in tensile strength of 10.5–17.1% in 10 weeks by *A. flavus, A. niger, A. parasiticus* and *R. oryzae* would place them in the category of inactive species (non-cellulolytic) according to Reese and Downing (1951). This implies that their growth and penetration of jute sack (without substantial decrease in tensile strength) would be a springboard for infecting grain content of sack.

From the mycological point of view, woven polypropylene is a superior packaging material since it neither supports fungal growth nor absorbs moisture and its tensile strength was not altered by fungi during 10 weeks storage at 90% R.H. Flatman (1977) stated that polypropylene sacks are much lighter in weight than jute sacks and the former has good tensile strength. Sacks made from polypropylene are clean and do not impart any odour or taint to their contents. There is also absence of contamination of loose hair fibres that support a de novo growth of fungi. Jute sacks on the other hand, absorb moisture rapidly and support prolific growth of fungi at high ambient humidity conditions (Fig. 1) to the detriment of the intrinsic tensile strength which was reduced by 50–75% in 10 weeks at R.H. 90% (Fig. 3). This implies that jute sacks under such high ambient R.H. conditions would not be able

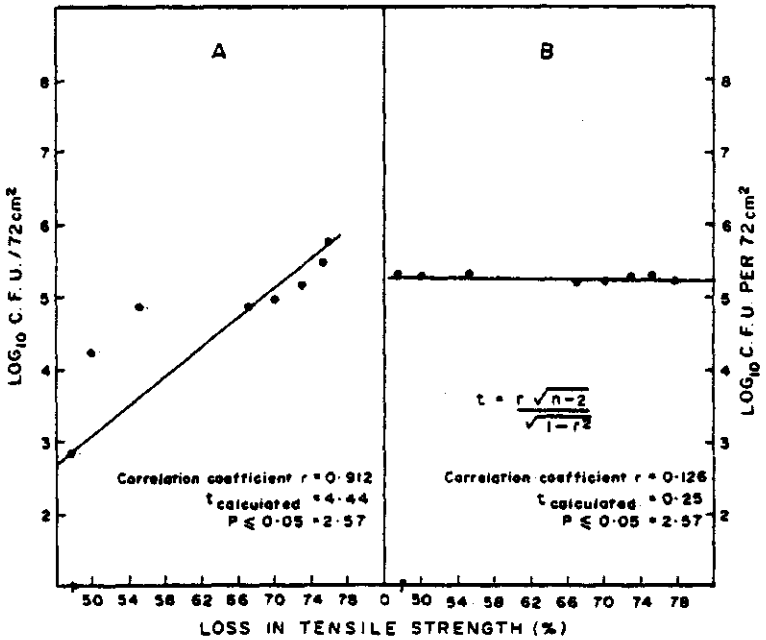

Fig. 3. Relationship between percentage loss in tensile strength of jute and mould and yeast counts (A) and total aerobic plate counts (B) after 10 weeks incubation at R.H. 90% and $30 \pm 2°C$.

to withstand rough warehouse handling and might break with increasing load. Indeed, this breaking or rupturing of jute sack is a common phenomenon in our warehouse facilities where grain foods are kept in jute sacks.

Open sacks with spilled food then become good food bait for fungi which shorten the shelf-life of the grains and alter the chemical quality of the grains by imparting mycotoxins to the food.

Both woven polypropylene and jute sacks can withstand a single gamma sterilization up to 1.0 Mrad (10 kGy) but woven polypropylene is one of the interesting materials for consideration because of its combination of inertness and mechanical properties making it useful for biomedical applications as well (Plester, 1970). Furthermore, for radurization in which the radiation dose does not exceed 1.0 Mrad (10 kGy), the Food and Drug Administration of the U.S.A. has approved the use of several packaging materials (polystyrene, wax coated paper board, nitrocellulose-coated cellophane, copolymer-coated cellophane etc.) including polypropylene for packaging of gamma-irradiated food (Anon, 1967). Currently, woven polypropylene sacks are in use for packing potatoes, vegetable seeds, metallic abrasives, fertilizers, sugar, coffee, rice, wheat and other crops exported to and imported from Europe and America. In Peru, woven polypropylene is used in fish meal trade for export markets. From our studies, the packaging of cereal grains and animal feed is another obvious future use of woven polypropylene sacks for storage of cereal grains and grain products in Ghana, and in other tropical countries.

However, the effectiveness of the woven polypropylene sack could be augmented by good storage practices and cleanliness of the warehouse.

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9. Influence of packaging material on moisture sorption and the multiplication of some toxigenic and non-toxigenic *Aspergillus* spp. infecting stored cereal grains, cowpea and groundnut

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The moisture sorption isotherms of jute sack were determined at 12, 22, and 32°C by a standard procedure using glycerol:water mixtures providing equilibrum relative humidities (ERH) of 65, 70, 75, 80, 90 and 95%. The equilibration period of jute sack kept at 65–85% was found to be between 6–8 days whereas the moisture content (MC) and weight of jute sack kept at 90–95% continued rising. The amount of moisture absorbed at 12°C was significantly greater ($P < 0.05$) than at 32°C. Analysis of variance applied to collected data of adsorption at ERH 85% (a_w 0.85) and desorption ERH 20% (a_w 0.20) showed that incubation period, type of commodity and type of packaging material as well as the interaction of these factors significantly influenced the moisture sorption and desorption by cowpea, groundnut (shelled), white maize, millet and sorghum. Each food commodity absorbed water differently in the jute and woven polypropylene sacks. However, food commodities stored in jute sack significantly absorbed and desorbed moisture to a greater extent than the same produce stored in woven polypropylene. Mould and yeast count on seed and grain lots in woven polypropylene at ERH 20% were 1.0–1.5 log cycles lower after 6 months storage than same seed and grain lots kept in jute sack. Correspondingly, mould and yeast count on seeds stored in woven polypropylene at ERH 85% were 2–3 log cycles lower than same produce kept in jute sack. Both ambient storage humidity and type of storage sack had profound effect on the occurrence of toxigenic and non-toxigenic mould species on cowpea, groundnut, maize, millet and sorghum. The toxigenic moulds and non-toxigenic *Aspergillus* species reduced considerably the shelf-life of seeds kept particularly in jute sacks. Practical implications of these findings are discussed in relation to grain food storage in the tropics.

Key words: Moisture sorption isotherms; Jute sack; Toxigenic fungi; *Aspergillus* spp., non-toxigenic; Cereal grains; Legumes

Introduction

In considering storage potential, the ambient equilibrum relative humidity (ERH) is an important parameter as it determines the amount of water available to microorganisms and hence is an indication of the biological activity or potential activity of the product (Ayerst, 1965). Above 75% RH, products absorb moisture rapidly and fungi develop rapidly during storage, and heating of the product would produce subsequent deterioration and loss of quality. RH below 75% is accepted as 'safe' for storage of food commodities e.g. cereal grains. A packaging material for cereal grains and legumes could either restrict passage of water vapour through the sack or may itself absorb and transfer moisture to its contents. A packaging material that would restrict passage of water vapour would also curtail increase and decrease in weight or moisture content of the enclosed product and could, therefore, extend the shelf-life of the food commodity.

The persistent use of jute sacks in packaging of cereal grains, pulses and other food commodities in Ghana has been attended by the loss of $\geqslant 30\%$ of annual harvest of crops due to insect infestation and growth of fungi. The pertinent literature has rarely any information on the moisture sorption of jute sacks under ambient tropical conditions and no comparative studies have been carried out to elucidate how water sorption of food commodities stored in jute sacks differ from the same commodities stored in other packaging materials such as woven polypropylene sacks.

The objectives of these studies were (a) to determine the moisture sorption isotherm of jute sack kept under pre-selected ERH values (65, 70, 75, 80, 85, 90 and 95% RH) (b) to examine the influence of packaging materials on moisture sorption at 85% ERH and desorption at 20% ERH of cowpea, groundnut (shelled), maize (white), millet, and sorghum stored in either jute or woven polypropylene sack – a suggested replacement for jute sack in tropical areas like Ghana (Odamtten and Kampelmacher, 1985a,b) (c) to study the multiplication of five potential mycotoxin-producing fungi (*Aspergillus flavus, Aspergillus ochraceus, Fusarium moniliforme, Penicillium digitatum* and *Penicillium expansum*) and five non-toxigenic *Aspergillus* spp. (*A. candidus, A. niger, A. terreus, A. sulphureus* and *A. panamensis*) under the moisture sorption humidity (85% RH) and desorption (20% RH) humidity conditions during storage for six months.

Materials and Methods

White maize (*Zea mais* L) millet (*Pennisetum americanum*) shelled groundnut, variety florispan runner (*Arachis hypogeae* L), cowpea red variety (*Vigna unguiculata* Walp.) and sorghum, local Accra red (*Sorghum bicolor* subsp. *bicolor*) were obtained from the market whilst unused jute and woven polypropylene sacks were supplied by the Grains Warehousing Company, Tema and the Polyproducts® factory, Accra, Ghana, respectively.

Moisture sorption isotherms of jute sack

This was determined by a standard method using glycerol:water mixtures to provide ERH values of 65, 70, 75, 80, 85, 90 and 95%. Seven desiccators (diameter 22 cm) were used to hold the humidity solutions. Forty sections of jute sack each weighing 5.0 g were placed at each RH inside the desiccators and were allowed to start moisture sorption at 32°C in a Freas 816 low temperature incubator (GCA/Precision Scientific Co., U.S.A.). The weights and moisture content of the samples were determined at regular intervals (2, 4, 6, 8, 10, 21, 28, 32 and 52 days) until they reached equilibrum. The moisture content was determined at each predetermined incubation period as outlined below.

Moisture content determination

The moisture content (%) of jute sack samples stored at ERH values between 65 and 95% were determined in duplicate before and after every indicated incubation period. The samples were dried at 103°C for 16 h. For maize, millet and sorghum, the International Standards Organisation method was employed (ISO 2291). Duplicate 10 g ground samples were dried at 103°C for 72 h. Cowpea and groundnut samples were dried at 113°C for 4 h in a mechanically ventilated Gallenkamp oven (Oxley et al., 1960).

Effect of temperature on moisture sorption by jute sack

To find out the effect of temperature on moisture sorption, quadruplicate 5 g samples of jute sack were stored at ERH of 65–95% and at temperatures of 12, 22 and 32°C for 14 days. The internal ERH values of the desiccators were adjusted by glycerol:water mixtures. After the equilibration period of 14 days the weight and the percentage moisture content of the jute sack strips were determined as outlined above.

Influence of packaging material on moisture sorption and desorption by food commodities

Four replicate samples (50 g) of cowpea, shelled groundnut, white maize, millet and sorghum were placed in satchets (10 cm × 5 cm) of either jute or woven polypropylene. The listed commodities in the two different packaging materials were then equilibrated to either ERH 85% for absorption, or to 20% for desorption at 32°C in desiccators where the ambient ERH was controlled by glycerol:water mixtures. The course of the moisture changes was followed by regular weighing of samples for up to 30–34 days.

Effect of packaging material and storage humidity on microbiological quality

Mould and yeast counts on naturally contaminated samples, initially, and after 2 and 6 months storage were estimated by pouring 10 g of the appropriate sample into 100 ml 0.1% peptone solution in 250 ml Erlenmeyer flasks which were subsequently shaken in a Gallenkamp Orbital shaker for 30 min at 140 rev/min. Serial dilutions were made from the stock suspension up to $1{:}10^6$. 1 ml spore suspension was cultured on oxytetracycline glucose yeast extract, OGYE (Oxoid CM 545). Characteristic colonies appearing after 3 days incubation at 28°C were counted. From this data the microbial loads, (log CFU g^{-1} sample) were calculated.

Estimation of germination capacity and length of emerging radicles

As an index of incipient deterioration owing to fungal activity in food commodities, the germination capacity of the samples was assessed using the blotter method of de Tempe (1967) and Limonard (1966). Seeds and grains with emerging radicles were considered to have germinated. The lengths of the emerging radicles of germinating seeds were measured for each storage sack stored at ERH values 20 and 85%. About 250 grains or seeds were used from each sack and storage humidity combination for this assessment.

Species composition and relative occurrence of fungi

Quantitative data on the number of fungal species isolated from the commodities and their relative occurrence was noted for all food commodities in the two types of packaging material. The relative occurrence of five toxigenic fungi (*Aspergillus flavus* NRRL 5906, *A. ochraceus* NRRL 6514, *Fusarium moniliforme* NRRL 6413, *Penicillium digitatum* Sacc. and *P. expansum* D19) and five non-toxigenic (*Aspergillus candidus. A. niger, A. terreus. A. sulphureus* and *A. panamensis* from the Centraal Bureau Schimmelcultuur, Baarn The Netherlands) was studied. These strains were similar, in cultural characteristics, to the local Ghanaian isolates.

Results

Moisture sorption isotherms of jute sack

In Fig. 1, results of the moisture sorption of jute sack are summarised. The equilibration periods of samples kept at ERH 65–85% were found to be between 6–8 days. The moisture content of jute sack kept at 90–95% ERH continued rising.

Effect of temperature on moisture sorption by jute sack

Fig. 2 summarizes the results obtained. Temperature significantly influenced the amount of moisture absorbed by jute sack ($P < 0.05$). Both the moisture content and weight of the sack at the lowest temperature used (12°C), were significantly higher ($P < 0.05$) than at the highest temperature used (32°C). Weight of sack and moisture absorption at 22°C lay between the two temperature extremes.

Influence of packaging material on moisture sorption by food commodities

The moisture sorption (85% RH) and desorption (20% RH) isotherms of cowpea and groundnut are summarised in Fig. 3, those for maize in Fig. 4 and for millet and

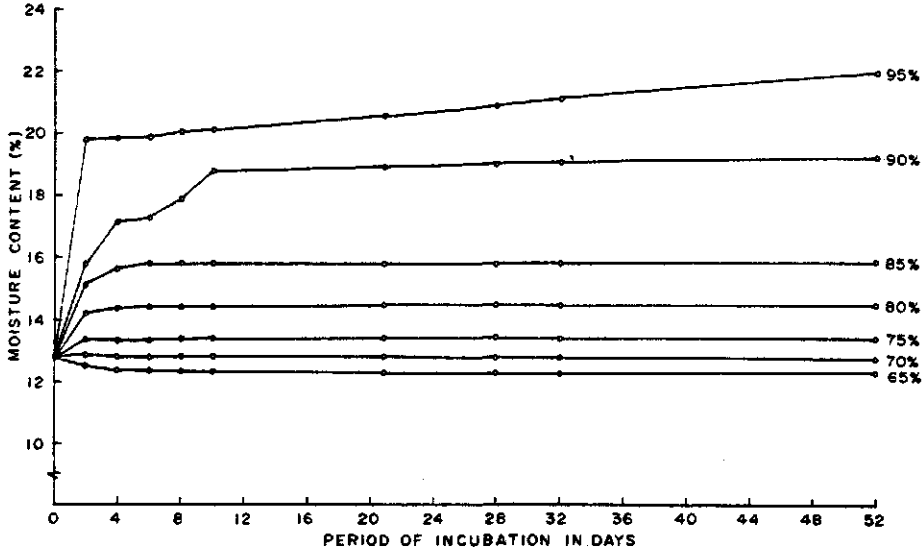


Fig. 1. Moisture sorption isotherms of jute sack stored at 32°C and the indicated humidities for 52 days.

sorghum in Fig. 5. Application of analysis of variance in order to examine the influence of incubation period (A), type of commodity (B) and type of packaging material (C), as well as the interaction of these factors showed that A, B and C

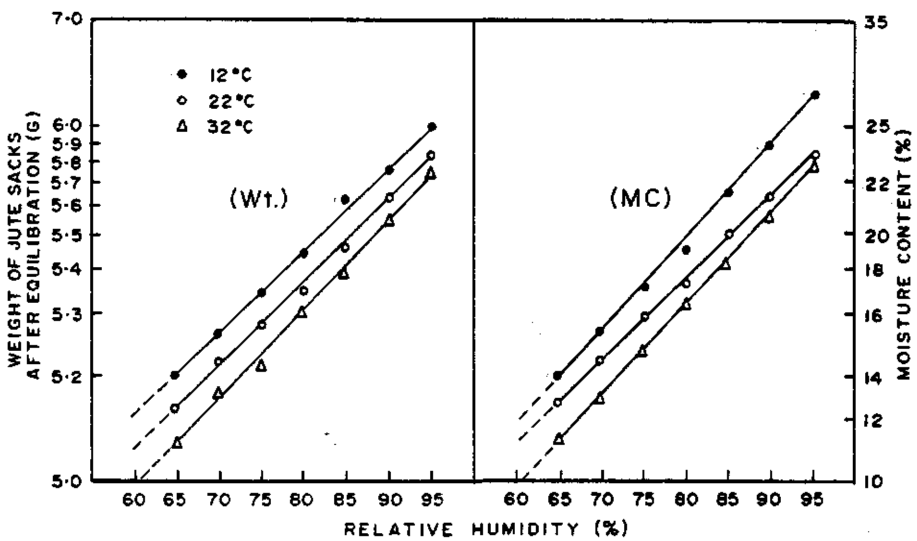


Fig. 2. Effect of storage temperature on weight and moisture content of jute sacks incubated for 14 days.

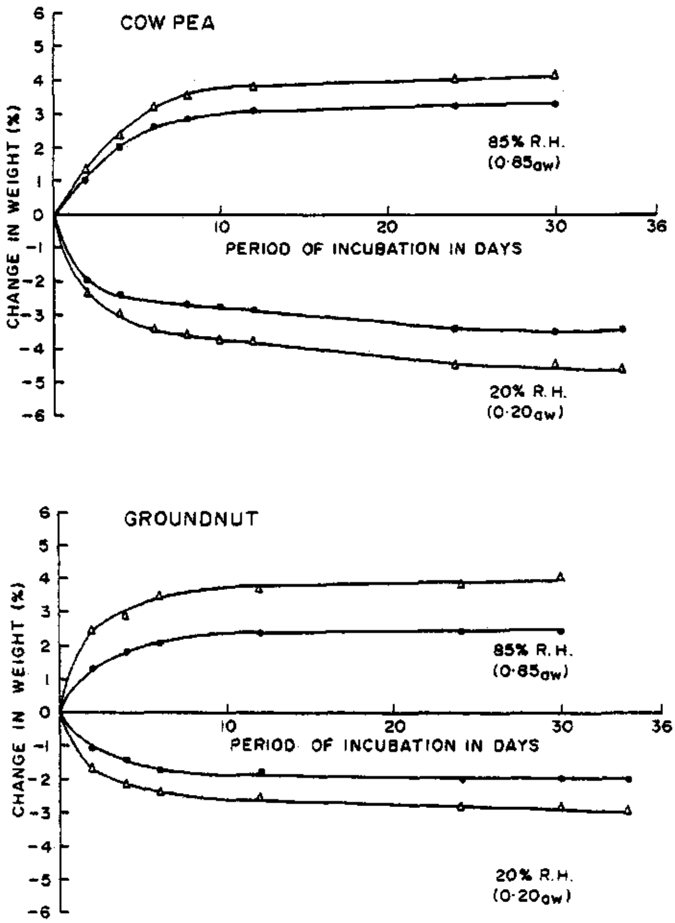


Fig. 3. The changes in weight of cowpea (top) and shelled groundnut (bottom) exposed to 20% and 85% ERH at 32°C for 30–34 days △——△ jute; ●——● woven polypropylene.

significantly ($P < 0.05$) influenced the moisture sorption and desorption by the five listed food commodities. However, interaction between A and B, A and C as well as B and C and A, B, C was highly significant ($P < 0.05$). Closer examination of the interaction of A and C showed that cowpea, groundnut, maize, millet and sorghum in jute sacks significantly absorbed and desorbed moisture to a greater extent than same produce stored in woven polypropylene sacks. For all food commodities water sorption differed according to the packaging material.

Microbiological quality

Ambient storage ERH and type of packaging material had an effect on mould and yeast count of food commodities. We found counts to be 0.5–1.0 log cycles

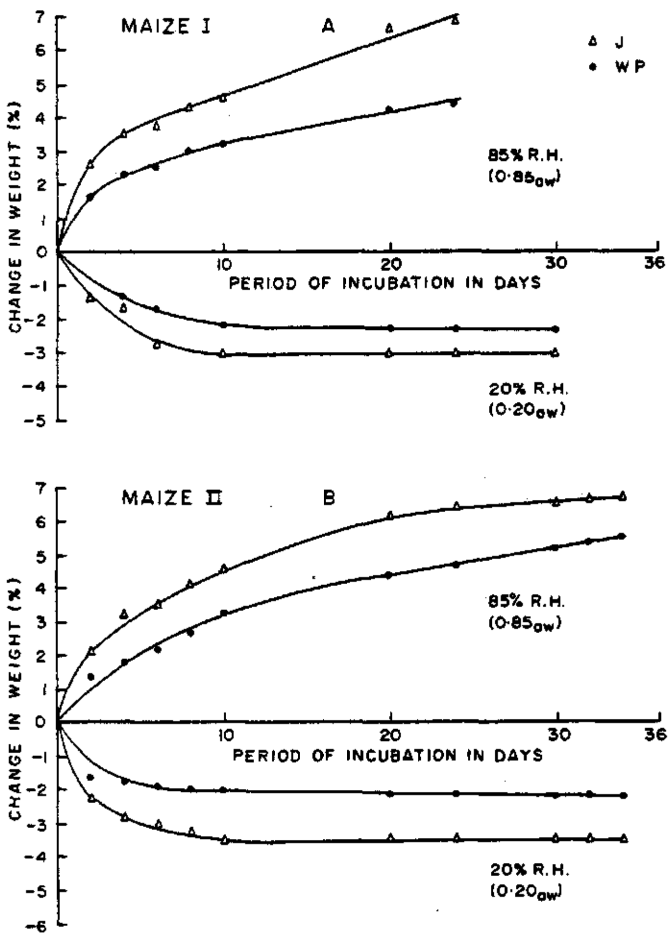
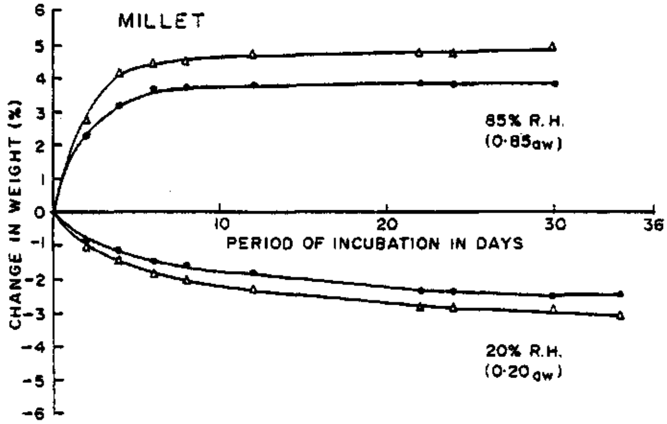


Fig. 4. The changes in weight of maize exposed to 20% and 85% ERH at 32°C. Maize II is a repeat experiment.

lower on commodities stored in woven polypropylene at 20% ERH for 2 months (Fig. 6A) than in jute sack and this difference increased to 1.0–1.5 log cycles after 6 months (Fig. 6B). Correspondingly, counts on commodities incubated at ERH 85% was 1–4 log cycles lower in woven polypropylene (depending on commodity) than in jute sack after 2 months storage, but this changed to 2–3 log cycles in 6 months (Fig. 6B). These observed differences were statististically significant ($P < 0.05$).

Both ERH and type of packaging material had a significant effect on the occurrence of toxigenic fungal species on the food commodities. Viable *A. flavus* spores occurred in cowpea, groundnut, maize and millet stored at both ambient 20% RH and 85% RH but not on sorghum. Groundnut was not infected by *A. ochraceus*



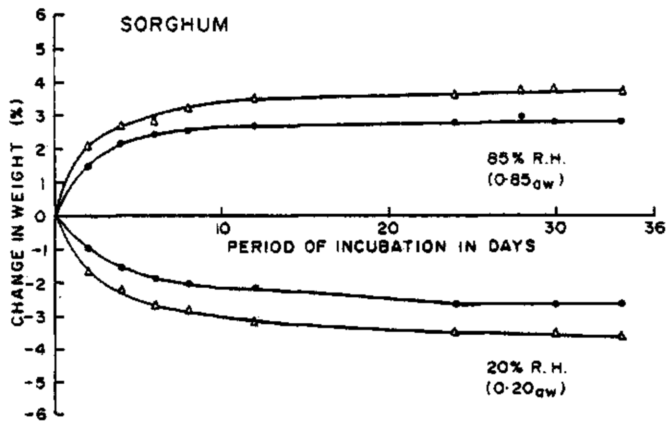


Fig. 5. The changes in weight of millet (top) and sorghum (bottom) exposed to 20% and 85% ERH at 32°C for up to 34 days.

whilst *F. moniliforme* infected all commodities stored at both 20 and 85% ERH except sorghum, groundnut, and cowpea which were not infected at 85% ERH. Only maize grains were not contaminated by *P. digitatum* (Fig. 7A). Finally, *P expansum* could not be isolated from all commodities stored in jute sack at 85% ERH but only maize, groundnut and millet stored at 20% ERH contained the spores of *P. expansum*. In all instances, significantly greater percentages of toxigenic fungi were recorded in commodities stored in jute sacks than in woven polypropylene sacks whilst only commodities stored in jute sack harboured toxigenic fungi (Fig. 7A). Infection by toxigenic fungi was reduced considerably after 6 months. (Fig. 7B).

Data for multiplication of non-toxigenic *Aspergillus* species on the five food commodities after 2 and 6 months storage are summarised in Fig. 7C and D. The

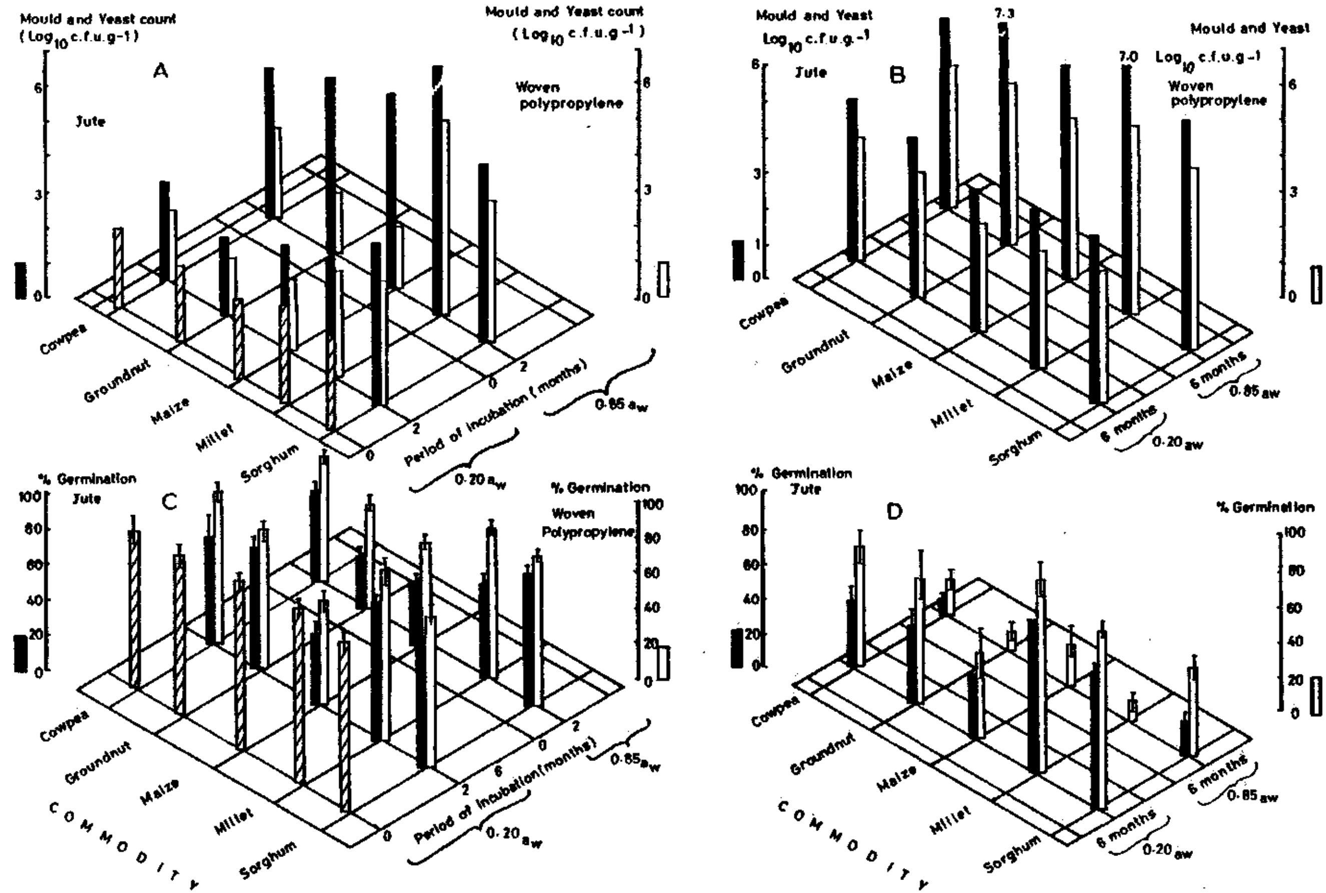


Fig. 6. Effect of the interaction between packaging material and ambient ERH on the mould and yeast counts and viability of seeds or grains of indicated food commodities. Mould and yeast counts on food commodities after 2 months (A) and after 6 months (B). Percentage germination (viability) after 2 months (C) and 6 months storage (D).

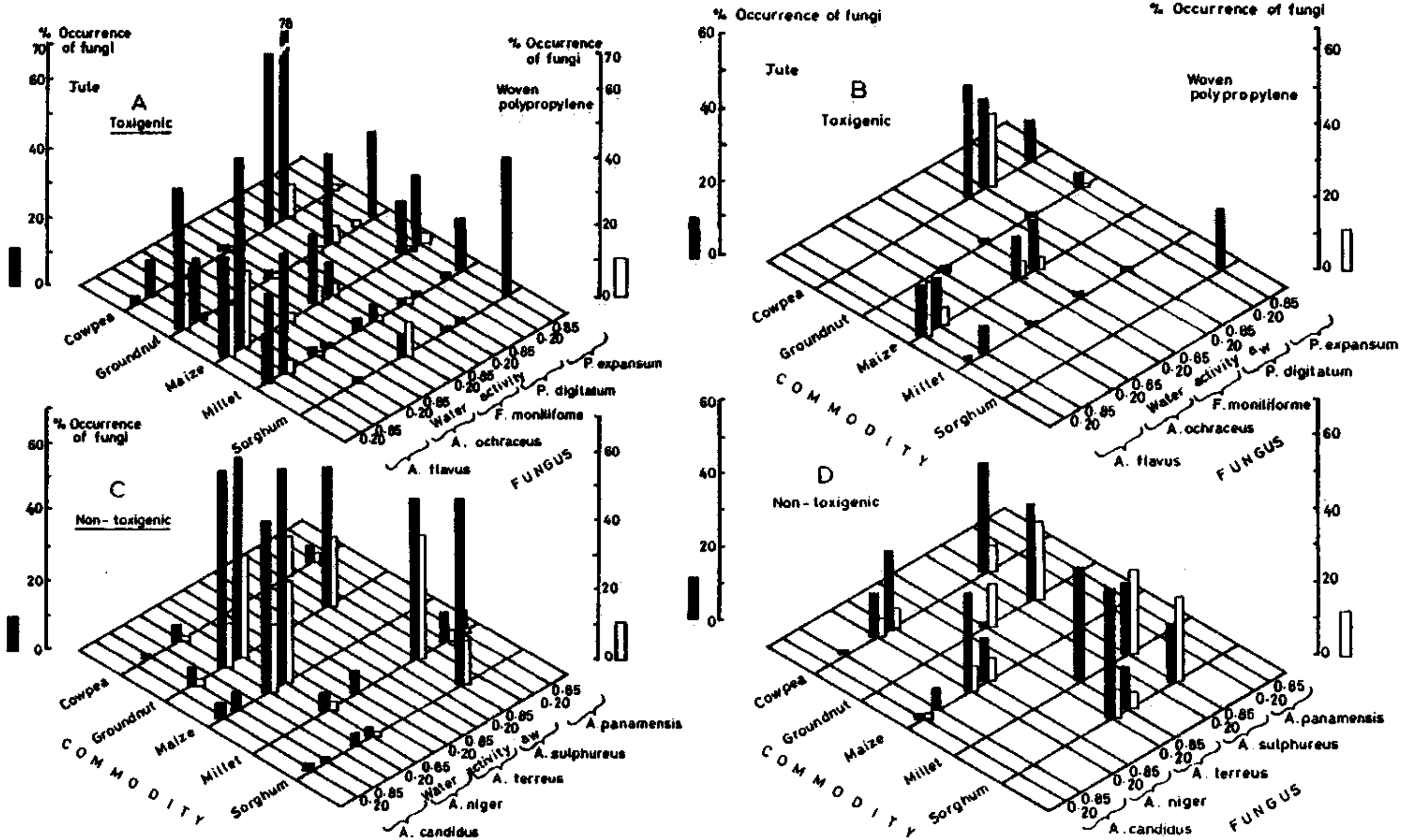


Fig. 7. Effect of packaging material and storage ambient humidity on the development of some toxigenic and non-toxigenic *Aspergillus* spp. in the indicated food commodities. Percent toxigenic fungal species isolated after 2 months (A) and after 6 months storage (B). Percent non-toxigenic fungal species isolated after 2 months (C) and after 6 months storage (D) period.

food commodities were infected variably with the different species of the non-toxigenic fungi. Mould occurrence was significantly higher ($P < 0.05$) in jute sacks than in woven polypropylene sacks (Fig. 7C). The populations of non-toxigenic *Aspergillus* species on the food commodities were higher at ambient ERH 85% than at 20%. After 6 months storage, the population of non-toxigenic moulds decreased in all instances so that *A. candidus* and *A. niger* could not be isolated from groundnut, millet and sorghum; *A. sulphureus* was not present on any food commodity stored at 20% ERH whilst *A. panamensis* could not be detected on all food commodities stored in both jute and woven polypropylene sack at both 20% and 85% ERH (Fig. 7D).

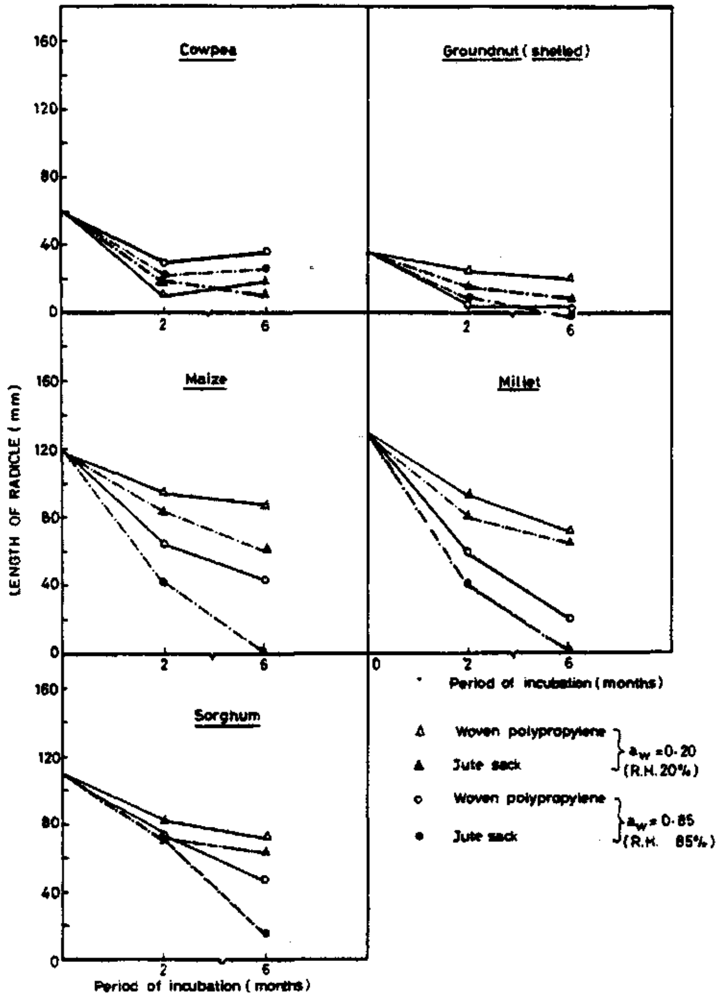


Fig. 8. Figure showing comparative changes in lengths of emerging radicles of germinating legumes and grains stored in either woven polypropylene or jute sack under ERH 20% and 85% for up to 6 months.

Germination capacity

Results obtained are summarised in Figs. 6C, 6D and 8. Both storage ERH and type of packaging material affected the germination of seeds and grains of the five food commodities as well as the length of the emerging radicles. Application of analysis of variance in order to examine the influence of type of commodity, type of storage sack, and period of incubation showed that the germination capacity of the grains and seeds differed according to packaging material. Percentage germination in jute sacks was significantly lower ($P < 0.05$) than that obtained in woven polypropylene sacks (Fig. 6C) and the emerging radicles were shorter in length than in the same seed lots stored in woven polypropylene sacks (Fig. 8). Seed lots of commodities stored at 20% ERH were statistically more viable than those incubated at 85% ERH ($P < 0.05$).

Moisture content

The decreases in moisture content of seed lots in jute sacks for six months at 20% ERH were as follows: cowpea (12.3 ± 0.7–8.2 ± 0.1%); groundnut (6.9 ± 0.1–5.4 ± 0.3%); maize (12.0 ± 0.6–11.6 ± 0.3%); millet (10.6 ± 0.2–8.4 ± 0.5%) and sorghum (9.0 ± 0.0–7.8 ± 0.2%). At 85% ERH moisture contents after six months for the five food commodities were as follows: cowpea (17.3 ± 0.1%); groundnut (10.2 ± 0.1%); maize (15.6 ± 0.3%); millet (16.8 ± 0.3%) and sorghum (17.8 ± 0.2%). Moisture contents of seed lots kept in woven polypropylene sacks were 0.8–2.3% lower than in jute sacks.

Discussion

Moisture sorption characteristics of jute sack (Fig. 1) were similar to those of maize grains kept under ERH 65–95% (Odamtten and Langerak, 1980). However, the amount of moisture absorbed by jute sack at 12°C was significantly larger ($P < 0.05$) than at 32°C. Hayakawa et al. (1978) also observed the same phenomenon in coffee products. Different products at same ERH may have different water contents (Lacey et al., 1980). Cowpea, groundnut (shelled), maize (white), millet and sorghum stored in jute sacks desorbed moisture to a greater extent than same seed lots stored in woven polypropylene sacks and this was reflected in the lower moisture contents recorded for seeds kept in woven polypropylene. Given the fact that each food commodity behaved differently, woven polypropylene is then a semi-barrier to moisture transfer to and from the sacks and the seeds.

Mould and yeast content on all food commodities stored at ERH values of 20% and 85% in woven polypropylene sacks were significantly lower than in jute sacks (Figs. 6 and 7). The viability of fungi isolated from the food commodities stored at low ERH 20% is not surprising. Although there was no visible growth of moulds, the spores remained viable and could commence a 'de novo' growth on suitable media. Eggins and Allsopp (1975) stated that quiescent growth of fungi into substrate enable growth and breakdown to continue even under adverse prevailing humidities at the surface. The presence of greater percentages of both toxigenic and non-toxigenic fungi on the food commodities stored in jute sacks than in woven polypropylene makes jute an inferior packaging material for the legumes (cowpea and groundnut) and the cereals (maize, millet and sorghum).

Germination is frequently referred to as one of the criteria upon which the condition of grains and seeds can be judged. In this paper, germination and viability are taken to be the same. Christensen and Kaufmann (1969) provided evidence that storage fungi are one of the main causes of loss of viability of seeds during storage. Storage fungi like *A. candidus*, *A. flavus*, and *A. restrictus* reduced percentage germination drastically from 97% to 13% or 0% in peas and maize respectively when stored for periods of 74 days up to 12 months (Lopez and Christensen, 1967; Fields and King, 1962). The critical moisture contents for safe storage of legumes and cereals in the tropics at 27°C and 70% ERH have been provided by Muckle and Stirling, (1971) as follows: cowpea, 15.0%; groundnut (shelled), 7.0%; maize (yellow), 13.0%; maize (white), 13.5%; millet, 15.0%; and sorghum, 13.5%. Our data suggest that at 20% ERH the moisture content of the seeds was on the 'safe' side in both woven polypropylene and jute sacks. However, storage at 85% ERH reduced drastically the viability and length of radicles of germinating seeds and increased the moisture content of seeds above the 'safe' level with the attendant increase of fungal deterioration particularly in jute sacks such that 0% germination was obtained for groundnut, maize and millet after 6 months storage in jute sack (Fig. 6C & D).

We conclude that, placed under the same ERH and temperature conditions, the five food commodities behaved differently in jute and woven polypropylene sacks. Each food commodity absorbed and desorbed moisture to a significantly greater extent ($P < 0.05$) in jute sack than in woven polypropylene. Woven polypropylene sack offered better protection from moisture than jute sack. The microbiological quality of the grains and seeds kept in woven polypropylene sack was better than in jute sack. This was reflected in the mould and yeast counts and the occurrence of toxigenic and non-toxigenic fungi. These fungi reduced the viability and length of radicles of seeds and grains stored in jute sacks for six months (Fig. 8). Woven polypropylene is therefore microbiologically and physically a better storage material for cereals and legumes (Odamtten and Kampelmacher, 1985a,b). We surmise that application of gamma irradiation and other treatments for mould decontamination of cereal grains and legumes in tropical areas like Ghana could be augmented by post-treatment storage of the food commodities in woven polypropylene sacks.

Acknowledgements

We are grateful to Mr. K.K. Etsibah of the Institute for Statistics, Social and Economic Research, ISSER, University of Ghana for computer analysis of data, Dr. S. Sefa-Dedeh, Department of Food Science, University of Ghana and Prof. J. Farkas, Central Food Research Institute, Budapest, Hungary for useful comments on the manuscript. We thank Mr. E.K. Barko for technical assistance and Mr. A.A. Brown for stencilling the graphs.

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10. SENSORY EVALUATION AND SOME QUALITY PARAMETERS OF MAIZE COMBINED-TREATED WITH HEAT AND GAMMA IRRADIATION

Abstract

This paper presents preliminary results of a consumer acceptance test performed with a popular Ghanaian Kenkey made from combined-treated corn dough presented to twenty adult panelists.

Krammer's Quick Rank Test showed that there was no significant difference ($P = 0.05$) in colour, flavour and taste between the control and the combined-treated maize grains. Starch viscosity measurements showed that the change in viscosity was not directly due to the combined heat and radiation treatments.

Heat-treated and combined-treated maize grains yielded more reducing sugars (as mg l^{-1} dextrose) than the unheated controls.

10.1 Introduction

Many consumers purchase or accept new products on the basis of sensory experience which it delivers. Sensory evaluation has been used for a long time to determine whether a new product matches some goals or target (Civille, 1978).

According to Blair (1978), the sensory evaluation process permits one to form a data-based point of view about your product. It gives you approximation of overall acceptability. Food irradiation stands in competition with other conventional means of food preservation processes and there is need to assess the acceptance of combined-treated grains in one of the 22 food preparations made from maize grains in Ghana.

10.2 Materials and methods

Batches of 1 kg maize grains were either heated at 60 °C for 30 min. in a specially designed heat-treatment chamber that enabled us to carry out the heating under either low (≤45% R.H.) ambient humidity or high (≥85% R.H.) ambient humidity. The two control samples were maintained at 20 °C for 30 min. under the prescribed humidities.

The grains were irradiated (within 30 min.) with 0 or 4 KGy of gamma radiation with a source dose rate of 0.06 KGy min.$^{-1}$.

The samples were soaked separately (1 kg in 1 litre of water for 48 hr) and then blended separately into flour. The resulting flours were wetted to

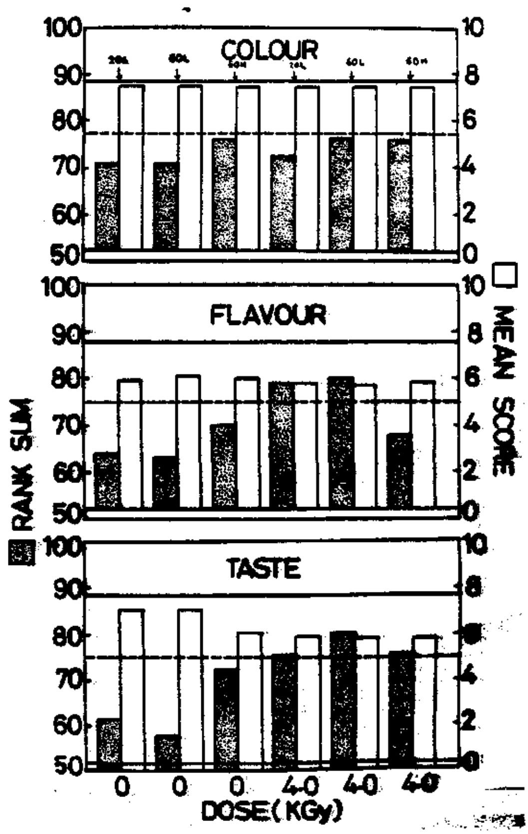

Fig. 1. Sensory quality of control and combined-treated maize grains. (Differences in rank sums between the lowest and highest limits, 52-88, are not significant at $P \leq 0.05$.)
----- Acceptability limit of mean score is 5.

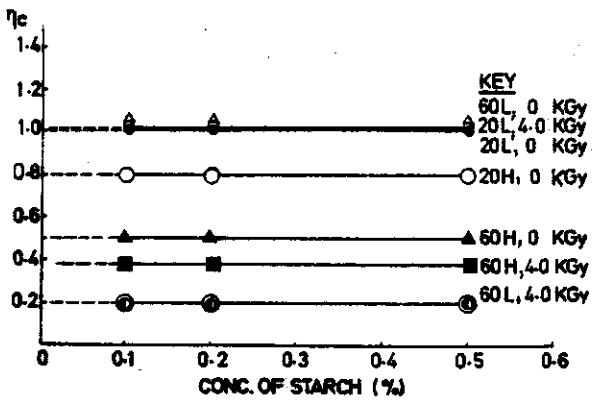

Fig. 2. Comparison between viscosity measurements of control and combined-treated samples.

form a dough and allowed to initiate fermentation at 25 ^{o}C for 48 hr. Thereafter, KENKEY, a popular Ghanaian meal was prepared using the local conventional method and the kneaded dough covered with 'corn leaves' boiled on a gas cooker for 90 min.

Coded samples representing all the treatment combinations were presented to the 20 panelists who were asked to judge the samples on a hedonic scale 1-10 (1-2 bad; 3-4 objectionable; 5 limit of acceptance; 6 medium; 7-8 good; 9-10 excellent). Results were assessed using Krammer's Quick Rank Sum Test.

Crude extract of maize starch was obtained from 100 g of blended samples previously soaked in water for 48 hr. The samples were dissolved in 1 litre of distilled water and then boiled for 20-30 min. before straining through cheese fine cloth. The maize starch solutions were diluted to form 0.1, 0.2, and 0.5% starch solutions and the viscosities of the starch were compared using an UBBELOHDE viscosimeter at 32 ^{o}C. The specific viscosities were calculated thus:

$$\eta_{sp} = \frac{t_s - t_w}{t_w}$$

in which

η_{sp} = specific viscosity
t_w = time for water to fall A to B 23.5 sec.
t_s = time for sample to fall from fixed point A to B.

$$\eta_c = \frac{\eta_{sp}}{\text{concentration of starch}}$$

The values of η_c obtained for each sample were plotted against concentration.

Reducing sugars (as dextrose) were estimated using Fehlings A and B solution according to the method of Lees (1968).

10.3 Results

There was no significant difference ($P = 0.05$) between colour, flavour and taste of control and combined-treated grains (Fig. 1). Differences in rank sums between the lowest and highest limits (52-88) were not significant at $P = 0.05$. The acceptability limit of mean score is 5.

The viscosity measurements showed that the combined-treated grains decrease in viscosity (Fig. 2). But the decrease in viscosity was not directly related to the heat and irradiation treatment.

The estimated reducing sugar content is presented in Table 1. Generally combined treated samples yielded more reducing sugars than the controls.

10.4 Conclusion

Maize grains combined-treated with heat and irradiation would be acceptable to the consumers. The decrease in viscosity which is reported to have brought about higher digestibility of grains is advantageous. Codex Alimentarius Wholesome Commission's acceptance as irradiated food subjected to overall dose of 10 KGy gives impetus to the practical application of our findings.

Table 1. Comparative reducing sugar yields of combined-treated maize grains starch.

Treatment	Dose applied (KGy)	Calculated value of reducing sugar as dextrose ($mg.l^{-1}$)
20 L	0.0	30.1
	4.0	31.7
20 H	0.0	31.6
	4.0	48.1
60 L	0.0	39.3
	4.0 *	66.1
60 H	0.0	52.3
	4.0 *	57.3

L = Low Humidity ($\leq$45% R.H.)
H = High Humidity ($\geq$85% R.H.)
* Combined treated samples
Calculated value of reducing sugar dextrose from pure Sample = 60.2 $mg.l^{-1}$.

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11. MICROBIOLOGICAL QUALITY AND PRODUCTION OF AFLATOXIN B_1 BY *ASPERGILLUS FLAVUS* LINK NRRL 5906 DURING STORAGE OF ARTIFICIALLY INOCULATED MAIZE GRAINS TREATED BY A COMBINATION OF HEAT AND RADIATION

G.T. Odamtten, V. Appiah, D.I. Langerak

Abstract

Maize grains artificially inoculated with 1.3×10^6 c.f.u/g of spores of *Aspergillus flavus* Link NRRL 5906 were kept in open containers either unheated (20 °C) or heat-treated (60 °C for 30 min.) under low humidiy (≤45% R.H.) or high humidity (≥85% R.H.) conditions and the grains subsequently irradiated within 30 min. in woven polypropylene sacks, with 0.0, 3.5, or 4.0 KGy. The grain samples were stored at 65% R.H. and 28 °C for 1 month and then at 80% R.H. for the following 3 months.

Moist heat treatment (60 °C for 30 min., ≥85% R.H.) does not increase significantly ($P = 0.05$) the initial moisture content of maize grains (above 13% m.c.) but reduced the initial mould and yeast count by 0.9 log cycles and the total aerobic bacteria count by 0.3 log cycles. A combination of moist heat and 4.0 KGy however, lowered the initial mould and yeast count by 5.1 log cycles and the total aerobic bacteria count by 4.2 log cycles. Incubation of 65% R.H. and 28 °C for 1 month augmented the killing effect of the radiation treatment. After 3 months storage at 80% R.H. the population of mould and yeast as well as the total aerobic bacteria of the combined treated grains (60 °C, 4.0 KGy) remained nearly the same (i.e. 5.0 and 4.3 log cycles reduction respectively from the initial) whereas control of the moist heat-treated grains had mould and yeast and total aerobic bacteria counts lowered by 1.5 and 1.3 log cycles respectively after 3 months storage at 80% R.H. The grains did not become rancid.

Based on the sensitivity of the method for aflatoxin B_1 detection, we found from triplicate samples that only control (20 L and 20 H) grains contained 0.8-4.0 µg/kg of aflatoxin B_1 after 3 months storage at 80% R.H. and 28 °C except the control (20 H) grains given 4.0 KGy.

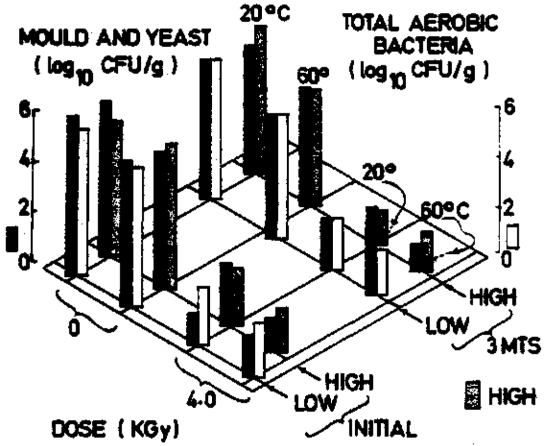

Fig. 1. Showing effect of the combination treatment on microbiological quality of maize grains.

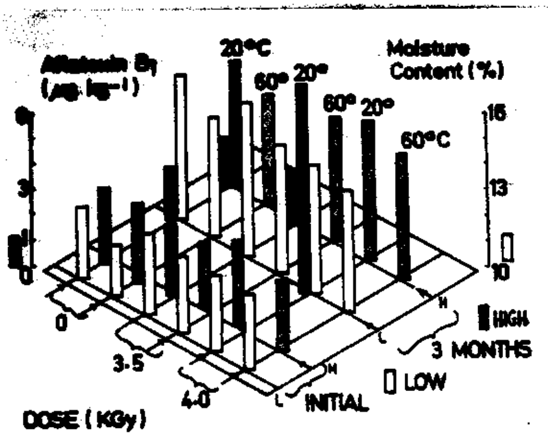

Fig. 2. Relationship between moisture content, combination treatment and level of aflatoxin B_1 formed in grains.

11.1 Introduction

The occurrence of *Aspergillus flavus* and aflatoxin B_1 in foodstuffs including maize and cassava flour has been reported in Ghana (Nartey, 1966; Lokko, 1978). Unavoidably, maize constitutes a major part of human diet in many countries.

Gamma irradiation in combination with heating has been proposed as an alternative means of extending the shelf-life of maize (Odamtten et al., 1980). Many researchers reported that gamma irradiation could increase aflatoxin formation (e.g. Applegate & Chipley, 1978; Priyardashini and Tulpule, 1976) in foods. Hence, we examined the aflatoxin content of combined-treated maize grains artificially inoculated with *A. flavus* spores and stored under tropical ambient conditions conducive for aflatoxin formation in maize.

11.2 Materials and methods

Maize grains (2 kg/beaker) were inoculated with 2.5×10^7 spores per ml of *A. flavus* by surface swabbing of grains and then covered with aluminium foil at 28 $^\circ$C for one day. Heating was performed in a specially designed heat-treatment chamber that enabled us to heat-treat the grain under either high humid conditions ($\geq$85% R.H., moist-heat) or under low humidity conditions ($\leq$45% R.H., dry heat) for 30 min. at 60 $^\circ$C. Unheated controls were maintained at 20 $^\circ$C for 30 min. After the heat-treatment 1 kg weight of grains were put in sachets of woven polypropylene sacks (30x15 cm) and the irradiation was carried out not more than 30 min. after the heat-treatment.
Gamma irradiation doses applied were 0.0, 3.5, and 4.0 KGy with a source dose rate of 0.06 KGy min.^{-1}.

The maize grains in the woven polypropylene sacks were stored at 80% R.H. and 28 $^\circ$C for four months. After every month, the moisture content (1SO 2291), aflatoxin B_1 content (Dantzman and Stoloff, 1972), rancidity (AOAC, 1960), mould and yeast (Mossel et al., 1970) and total aerobic bacteria (1SO 4833) were determined.

11.3 Results

Moist heat treatment (60 $^\circ$C for 30 min., $\geq$85% R.H.) does not increase significantly the initial moisture content of maize grains (about 13% m.c.) but reduced the initial mould and yeast count by 0.9 log cycles and the total aerobic bacteria count by 0.3 log cycles (Fig. 1). A combination of moist heat 60 H and 4.0 KGy however, lowered the initial mould and yeast count by 5.1 log cycles and the total aerobic count by 4.2 log cycles (Fig. 1).

After 3 months storage at 80% R.H. the $\log_{10}$ c.f.u./g count of mould and yeast and total aerobic bacteria of the combined treated grains (60 H, 4.0 KGy) remained nearly the same (i.e. 5.0 and 4.3 log cycles reduction respectively from the initial), whereas control of the moist heat treated grains had mould and yeast and total aerobic bacteria counts lowered by 1.5 and 1.3 log cycles respectively after 3 months storage at 80% R.H. The grains did not become rancid.

We found from triplicate samples that only control (20 L and 20 H) grains contained 0.8-4.0 µg/kg of aflatoxin B_1 after 3 months storage at 80% R.H. and 28 °C (when soaked for 2 days before extraction) except the control grains given 4.0 KGy (Fig. 2).

11.4 Conclusions

We conclude that:

(i) A combination treatment of moist heat (60 °C applied for 30 min. under $\geq$85% R.H.) and 4.0 KGy can improve the microbiological quality of grains kept at 80% R.H. and 28 °C in woven polypropylene sacks by 4-5 log cycles.

(ii) Combined-treated (60 H, 4.0 KGy) *A. flavus* spores could not form aflatoxin B_1 in the variety of yellow maize used.

(iii) Maize varietal differences influence their susceptibility to aflatoxins contamination and therefore further studies using other local Ghanaian maize varieties should be pursued.

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12 DISCUSSION

Comparison of mycoflora recorded on maize with that obtained from other agricultural products in relation to their spoilage potential

In Table 1 is summarized mycoflora on maize grains isolated in Ghana (Article 1) as compared with that found on other cereal grains, legumes and grain products.

Microorganisms have been known to infect stored grains, legumes and oilseeds as well as flours and further-processed food produced from them. Microbial invasion of stored grains and grain prouducts can result in various kinds of damage. Fungi decrease viability of seeds (hence reducing crop production potential) cause discolouration (which reduce grade) and therefore monetary value of grains and other seeds.
Microbes are primarily responsible for the heating of moist grains and legumes. Mustiness, caking, total decay are the final stages of spoilage. Musty odours persist throughout whatever processing treatment the infected grains and seeds may be subjected to during the preparation of food (Beuchat, 1978).

The carcinogenic, mutagenic and teratogenic effects of mycotoxins produced by storage fungi is a problem of grave concern, for their production *in vivo* is a serious threat to wholesomeness of foods prepared from infected grains.

Mould growth depends on moisture content of seeds, temperature, humidity, rapidity of drying, aeration, the microbiological ecosystem, insects, mixing of seeds, chaff and dirt, chemical treatment, internal infection, accidental rewetting of the seeds by condensation or leakage and the development of hot spots (Hesseltine, 1976).

The mould contaminants encountered in Article 1 and those in Table 1 belong predominantly to the genera *Aspergillus* and *Penicillium*. Minimum temperatures for growth and toxin production of Aspergilli are higher than those of Penicillia. The water activity, a_w, for growth of Penicillia differs from those of Aspergilli (Northolt, 1979). One of the most important factors influencing fungal growth on grains, legumes and oilseeds is moisture content (m.c.). The minimum m.c. for storage of grains and legumes in the pertinent literature is summarized in Table 2. Northolt (1979) stated the conditions for growth and production of some mycotoxins formed by *Aspergillus* spp. and *Penicillium* spp. Mycotoxins are not formed until the mould has gone through logarithmic growth phase. For example, aflatoxin is not formed until at least 48 h after spores of

Table 1. Comparison of mycoflora of maize grains in Ghana with what exists on other cereal grains, legumes and seeds.

Commodity	No. of fungi isolated belonging to the germs		Total Nº of fungi	Potential mycotoxin-producing species			
	Aspergillus	*Penicillium*		*A. flavus*	*A. ochraceus*	*F. moniliforme*	*P. digitatum* *P. expansum*
Maize	10	5	42	+	+	+	-/+
Millet	9	4	23	+	+	+	+/+
Sorghum	6	3	15	-	+	+	+/+
Rice	7	4	10	+	+	-	+/+
Cowpea	6	2	37	+	-	+	+/-
Cottonseed	7	4	18	+	+	+	+/+
Bambarra groundnut	2	2	7	+	-	+	-/-
Groundnut (Florispan runner variety)	10	13	38	+	+	+	+/+
Groundnut (Kumawu red variety)	10	13	43	+	+	+	+/+

References: Maize: Danquah, A.-O., 1973; Odamtten, G.T., 1981.
Millet: Danquah, A.-O., 1973; Odamtten, G.T., and E.H. Kampelmacher, 1986.
Sorghum: Danquah, A.-O., 1973; Odamtten, G.T., and E.H. Kampelmacher, 1986.
Groundnut: Markwei, C.M., 1976.
Bambarra groundnut: Danquah, A.-O., 1973.
Cotton seeds: Odamtten, G.T. and G.Y.P. Klu, 1987.
Rice: Danquah, A.-O., 1973.
Cowpea: Danquah, A.-O., 1973; Odamtten, G.T., and E.H. Kampelmacher, 1986.

Aspergillus flavus germinate (Hayes et al., 1966). High ambient moistures and temperatures characteristic of the tropical climate ($\geq$80% R.H., 28±3 ^{o}C) hasten the microbial deteriorative process.

According to Fig. 2 in Article 1 there were four infection patterns in the mycoflora encountered. This implies that in certain instances, the fungus may form the toxin in the grain but may not be isolated later on with prolonged storage time. This microbial competition which inhibits growth and mycotoxin production has been demonstrated by other workers (Ashworth et al., 1965; Wicklow et al., 1980). But once mycotoxins are formed, removal or destruction in seed, grain or grain products is difficult and often expensive. Mycotoxins known to occur naturally in grains, legumes or oilseeds are summarized in Table 3.

Hesseltine (1976) enumerated some of the problems associated with detoxification of commodities:

(a) Any chemical of physical treatment that removes mycotoxins adds to the cost of the product already damaged by mould growth; besides the initial processing cost, additional material may be lost from separating the toxin-infected parts mechanically or chemically;

(b) Most processes that remove mycotoxins are not 100% efficient;

(c) When chemical processes are used, extensive testing is required to establish that a second biologically active compound with a different mode of action has not been formed;

(d) Processes that remove mycotoxins may reduce the food value of the final product.

Marth and Doyle (1979) updated the various processes used in the degradation of aflatoxin in foods and they concluded that much additional research is needed before this becomes practical.

Although chemical sterilants for the control of post-harvest diseases of insects and fungi are well known, they do not kill fungi (Article 7), in addition, the chemical residues which they impart to foods leave much to be desired. In view of this, public health authorities the world over have stringent regulations on the reduction or elimination of chemical burdens in food. It is, therefore, expedient to prevent growth (and toxin formation), of moulds before chemical damage is done. The different food commodities listed in Table 1 share common storage fungi and this could be a basis for extending results obtained for maize to these agricultural products.

Table 2. Moisture content and corresponding water activity (a_w) of seeds at 25-30 °C.

Water activity (a_w)	% Moisture content of named commodities							References[a]
	Maize/Sorghum	Rice rough	Rice polished	Millet	Cotton seed	Cowpea	Groundnut (shelled)	
0.65	12.5 - 13.5	12.5	14.0	-	8.5	-	6.2	Christensen, 1972
0.70	13.5 - 14.5*	13.5	15.0*	15.0*	10.0*	15.0*	7.0*	Food & Nutrition Paper No. 10, FAO 1979
0.75	14.5 - 15.5	14.5	15.5	-	11.2	-	7.8	Muckle & Stirling, 1971
0.80	15.5 - 16.5	15.0	16.5	-	12.5	-	10.3	Christensen, 1972
0.85	18.0 - 18.5	16.5	17.5	17.8	14.5	17.3	12.2	Christensen, 1972; Majumber et al., 1965

* Maximum moisture content (m.c.) for safe storage.

a: other references: Maize: Pixton and Warburton, 1971a.
Sorghum: Ayerst, 1965.
Cotton seed: Navarro and Paster, 1978.
Groundnut (shelled): Pixton and Warburton, 1971b.

Interaction between storage humidity and gamma irradiation or its combination with heat

The application of ionizing radiation as a processing method offers a better alternative to chemical insecticides and fumigants because application of ionizing radiation leaves no chemical residues and no insect or fungus has been found to develop immunity to irradiation. In Figs 1 and 2, complete control of fungi on maize could not be obtained even with a dose of 5.0 KGy because high environmental storage humidity (R.H. $\geq$80%) increased radio-resistance of the spores of *A. flavus* (Odamtten, 1979; Amoaka-Atta et al., 1981) by making more free water available to spores for growth after the radiation treatment. Radiation up to 5.0 KGy only delayed the onset of infection. For example, probit analysis of data in Appendix 1 shown in Figs 1, 2, and 3 indicate thus: 84% spoilage = $\log_{10}$ 1.5 days ($\approx$4.5 days) at 85% R.H.

84% spoilage at 85% after applying 5.0 KGy =

= $\log_{10}$ 1.9 days ($\approx$6.7 days).

The higher the storage humidity, the faster the rate of spoilage of grains.

The heat treatment apparatus in Article 2 offered the possibility of augmenting the killing effect of irradiation by first enabling us to apply moist (at $\geq$85% R.H.) or dry (at $\leq$45% R.H.) heat (60 ^{o}C for 30 min.) to grains prior to irradiation. This synergistic effect lowered the gamma irradiation dose required to 4.0 KGy and the treatment was more effective after exposing spores to moist heat (60 ^{o}C for 30 min., $\geq$85% R.H. ambient humidity) before irradiation (Fig. 3) than when dry heat (60 ^{o}C for 30 min., $\leq$45% R.H. ambient humidity) was applied during the heating of grains.

Experimental results in Article 3 show that spores of *A. flavus* NRRL 5906 irradiated in the wet state were more radiation sensitive than those treated in the dry state. For, indeed, whilst a sublethal temperature of 53 ^{o}C applied for 5 min. in combination with 0.75 KGy inactivated moistened spores suspended in Tween-80 solution, a dose of 4.0 KGy was required in combination with prior heating (60 ^{o}C for 30 min.) under moist humidity conditions ($\geq$85% R.H.) to achieve the same objectives for dry *A. flavus* spores. Thus fruits and vegetables which can be immersed in hot water would require much lower doses to kill contaminating spoilage fungi than dry grains infected with the same fungal species. The reason could be that the enlarged volume of spores in water is attended by enhanced metabolic processes which are vulnerable to heat and low gamma irradiation. Indeed, according to Gregory (1966), dry spores have low water content and slow metabolism, lack vacuoles and cytoplasmic movement.

Table 3. Mycotoxins and organisms producing toxins in various cereal grains and seeds.

Mycotoxin	Organism	Foodstuff affected	References
Aflatoxins[a]	*Aspergillus flavus* *A. parasiticus*	Groundnut, rice, Maize, Cotton seed Coconut, Cocoa beans Wheat, Millet, Sorghum	1 2 3
Aspergillic[a] acid	*A. flavus*	Cereals	1
ATA toxin	*Fusarium sporotrichoides*	Oat, Wheat, Barley	4
Ochratoxin[a]	*Aspergillus ochraceus* *P. viridicatum*	Cereals	1, 5, 6, 7, 8, 9,
Citrinin[a]	*Penicillium citrinum* *P. chrysogenum*	Rice Cereals	 1, 10
Penicillic acid[a]	*P. cyclopium* *P. puberulum*	Maize	1
Patulin[a]	*P. expansum,* *P. urticae* *P. patulum* *P. digitatum,* *Byssochlamus,* *A. clavatus nivea*	Maize, Rice Animal feed	1, 5, 6
Rubratoxins	*P. rubrum* *P. purpurogenum*	Maize, Cereals Meat	 1
Sterigomato-cystin[a]	*A. versicolor* *Aspergillus nidulans*	Cereals	11, 12, 6
Moniliformin[a]	*Fusarium moniliforme*	Maize, Cereals, Legumes	1, 5
Roquefortine[a]	*Penicillium commune* *P. roqueforti*	Cotton seed	13, 14
Zearalenone[a]	*Fusarium graminearum*	Maize, Hay, etc.	1, 15
Kojic acid[a]	*A. flavus* and other *Aspergillus* spp.	Cereals	1
Penitrem A	*Penicillium commune* *P. crustosum*	Cotton seeds	16
T-2 Toxin[a]	*Fusarium tricinctum* *F. nivale, F. poae*	Cereals, Maize	1
Butenolide	*F. nivale*	Maize, cereals	1
Tremorgenic toxin	*A. flavus*	Maize and other foodstuffs	1
Beta-nitro propanoic acid	*A. flavus*	Cereals	1

Table 3 (continued)

Mycotoxin	Organism	Foodstuff affected	References
Nivalenol[a]	*Fusarium nivale*	Rice, Cereals	1
Deoxynivalenol[a]	*Fusarium nivale*	Rice, Cereals	1
Fusarenone	*Fusarium nivale*	Rice, Cereals	1

a. Mycotoxins detected as natural contaminants.

References: 1 - FAO, Food and Nutrition Paper No. 10, 1979.
2 - Widstrom, 1979.
3 - Zuber and Lillehoj, 1979.
4 - Ciegler et al., 1972
5 - Odamtten, 1981.
6 - Reiss, 1978.
7 - Harwig et al., 1974.
8 - Krogh, 1974.
9 - Pavlovič et al., 1979.
10 - Austwick, 1975.
11 - Hitokoto et al., 1978a.
12 - Hitokoto et al., 1978b
13 - Ciegler, 1969.
14 - Ohmomo et al., 1977.
15 - Scott and Kennedy, 1976.
16 - Waganer et al., 1980.

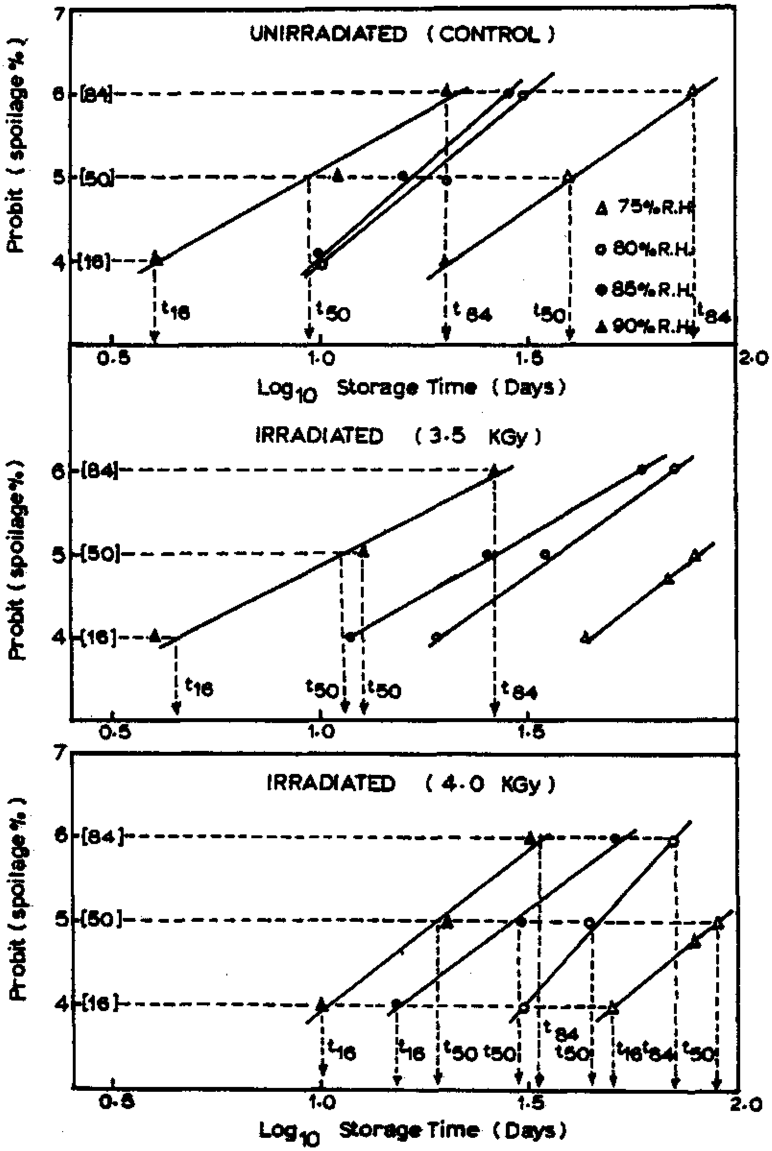

Fig. 1. Probit analysis of percentage spoilage data of maize grains stored at indicated relative humidities (R.H.) after irradiation with 0.0, 3.5, and 4.0 KGy. (Percentage spoilage data are in parenthesis [ ]; see Appendix Figs 1 and 2.)

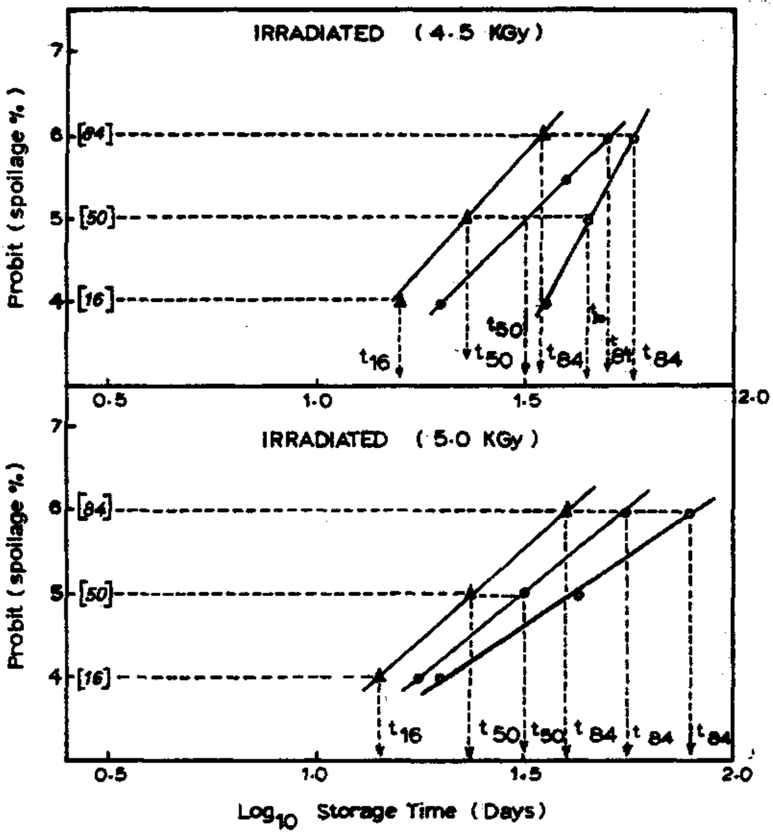

Fig. 2. Probit analysis of percentage spoilage data of maize grains stored at indicated relative humidities (R.H.) after irradiation with 4.5 and 5.0 KGy. (Percentage spoilage data are in parenthesis [ ]; see Appendix Figs 1 and 2.)

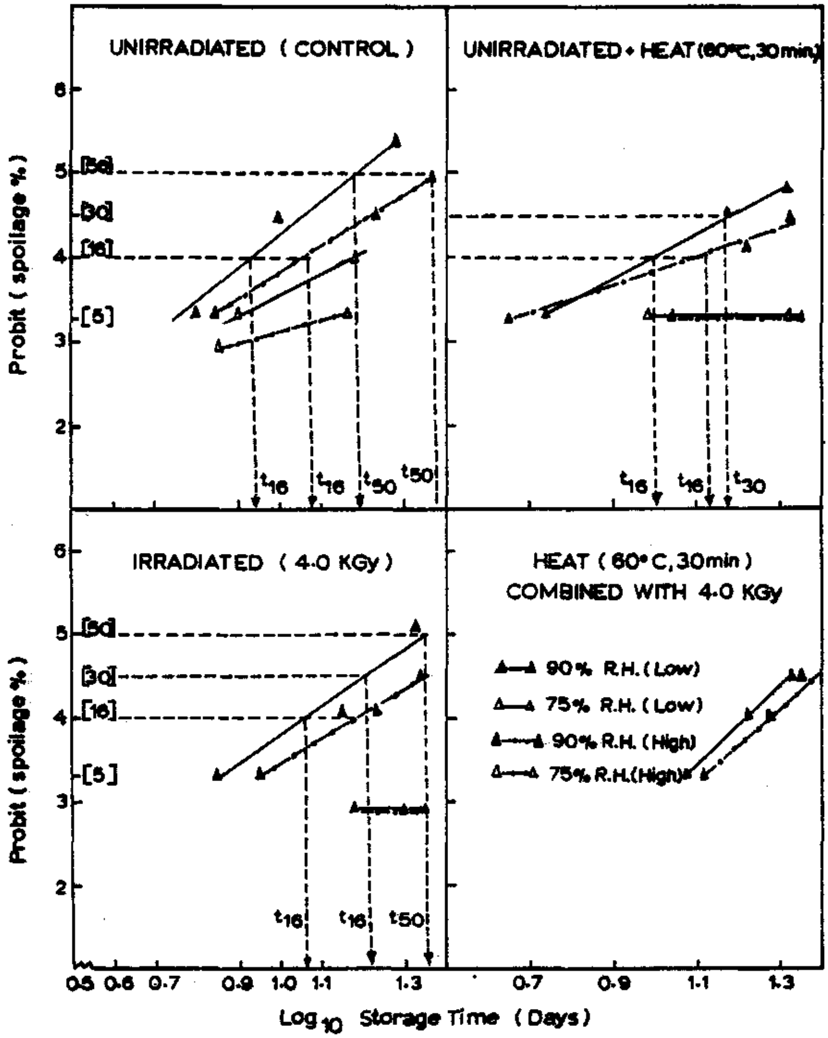

Fig. 3. Probit analysis of percentage spoilage data of maize grains stored at indicated relative humidities (R.H.) after heating and its combination with gamma irradiation. (Note the absence of infection at storage humidity $\leq 80\%$.)

However, they contain enzymes for a variety of metabolic activities (Knight, 1966). Before swelling (and protrution of germ tubes) spores are 3-10 times more active than mycelium (Vézina et al., 1968). When hydrated, ungerminated spores have their enzymes activated and they contain if not all the components of protein synthesis apparatus (ribosomes, t-RNA, aminoacyl t-RNA synthetase). Heating would "sensitize" the spores and render them more vulnerable to low radiation ($\geq$0.75 KGy). Radiation would impair the m-RNA already present in hydrated dormant spores; which would have acted as template for protein synthesis in the formation of new wall of germ tube (Brambl and Van Etten, 1970; Van Etten, 1969). We could not explain from data in Article 3 the recovery of heated (60 ^{o}C for 30 min.) and unirradiated dry spores of *A. flavus* after storage at 80% R.H. for 8 days. Further investigations are needed to explain these observations.

Effect of combination treatment on aflatoxin formation

Aflatoxins are regarded as the most potent carcinogenic, mutagenic and teratogenic mycotoxins of human and animal health importance (Goldblatt, 1969; Maggon et al., 1977; Heathcoate and Hibbert, 1978). Two economically important aflatoxin-producing strains *Aspergillus flavus* and *Aspergillus parasiticus* are ubiquitously associated with a wide variety of stored commodities (Diener and Davis, 1977). The fungus *A. flavus* is the most radioresistant fungal species associated with stored grains, legume and oilseeds and this makes it a reasonable choice for determining the effect of the combination process on mycotoxin production. Any effective control measure for *A. flavus* growth and toxin formation could be extended to cater for other radiosensitive species. Table 4 summurizes pertinent literature on mycotoxin production by either *A. flavus* or *A. parasiticus* before and after exposure to gamma irradiation. Doses of 4.0 KGy or more prevented spore germination entirely, hence no aflatoxin was produced (Applegate and Chipley, 1974a).

Results in Article 4 show that the nature and type of heat applied to spores influenced growth and amount of aflatoxin formed. Aflatoxin B_1 formation be *A. flavus* spores incubated in ammended maize meal broth (AMMB) was completely prevented by a combination of moist heat (60 ^{o}C for 30 min.) applied under 85% R.H. prior to exposure of spores to 4.0 KGy of gamma irradiation but not when treated in the same way with dry heat prior to irradiation. Heat treatment too has been proved to be mutagenic (Eigner et al., 1961; Northrop and Slepecky, 1967; Bridges et al., 1969). But it is well known that high humidity strikingly

Table 4. Production of aflatoxin B_1 by *A. flavus* and *A. parasiticus* before and after gamma irradiation or its combination with heat.

Treatment dose (KGy)	*A. flavus* NRRL 3145[a]	*A. flavus* NRRL 5906[b]		*A. parasiticus* NRRL 2999[c]		*A. parasiticus* NRRL 3000[c]	
	Synthetic medium (µg)	MMB (µg ml^{-1})	AMMB (µg ml^{-1})	Spores (µg mg^{-1})	Mycelium (µg mg^{-1})	Spores (µg mg^{-1})	Mycelium (µg mg^{-1})
0.0	0.18 -0.029	0.0- 2.0	0.0- 4.0	2.9	2.6	1.3	0.6
1.0	0.035-0.170	*	*	3.6	0.7	1.1	0.3
2.0	0.005-0.057	*	*	3.1	0.3	0.7	<0.1
3.0	0.005-0.012	*	*	*	*	*	*
3.5 + Dry heat	*	32.0-57.0	8.0-70.0	*	*	*	*
3.5 + Moist heat	*	6.0-10.0	4.0-45.0	*	*	*	*
4.0	ND	15.0-49.0	5.0-12.0	*	*	*	*
4.0 + Dry heat	*	4.0-20.0	15.0-20.0	*	*	*	*
4.0 + Moist heat	*	ND	ND	*	*	*	*

a: Applegate and Chipley, 1974a.
b: Odamtten, Appiah and Langerak, 1985a; Odamtten, Appiah and Langerak, 1985b.
c: Bullerman, Barnhart and Hartung, 1973.
*: Data not available in pertinent literature; ND: Not Detected; MMB: Double autoclaved maize meal broth; AMMB: Maize meal broth ammended with glucose and peptone.

affects lethality of heat applied. Moist heat shows greater lethality because (a) enzymes are more readily coagulated and hydrated, (b) heat is transferred more readily in wet air (Hawker et al., 1952).

Enhanced aflatoxin production after gamma irradiation by *A. flavus* and *A. parasiticus* has been reported by many workers (Applegate and Chipley, 1973, 1974a,b; Bullerman et al., 1973; Jemmali and Guilbot, 1969, 1970a,b; Priyadarshini and Tulpule, 1976; Schindler et al., 1980). This enhanced aflatoxin production has been explained on the basis of mutations resulting from exposure of spores to radiation. The general mutational effect of irradiation evidently causes damage in genetic and biochemical mechanisms, creating impairments of function and biological demands where none existed before (Ingram and Farkas, 1977). While this is likely to make organisms more difficult to grow and recognize, it does not seem likely to make them more pathogenic, rather the reverse.

Irradiation sometimes increases toxin production and sometimes diminishes it. There were no clear indications as to why the one or the other should happen on a particular occasion; generally such behaviour was expected on the basis of random mutation (Ingram and Farkas, 1977).

However, results in Article 5 show that reduction in number of spores of *A. flavus* by 3-4 log cycles either by serial dilution, heating or heating in combination with irradiation led to manifold (3-12 fold) increase in aflatoxin B_1 formed by the spores. The aflatoxigenic potential of *A. flavus* was inversely proportional to the inoculum size: high inoculum size of spores yielded the least aflatoxin. Sharma et al., 1980) reported that for *A. parasiticus* spores, irradiation (and its combination with heat) may not have any direct effect on aflatoxin production but on the size of inoculum in relation to viable spores. This confirms findings in Article 5. Moist heat (60 ^{o}C applied for 30 min. under $\geq$85% R.H.) combined with 4.0 KGy completely impaired the aflatoxigenic potential of *A. flavus* spores.

Influence of other factors on the formation of aflatoxin

Rather curiously, *A. flavus* spores failed to form aflatoxin in culture on maize meal broth (Article 4) except when the medium was autoclaved twice at 121 ^{o}C for 15 min. The excessive heating possibly released mineral elements required for aflatoxin biosynthesis. Information in the literature shows that autoclaved media support production of greater amount of aflatoxin than non-autoclaved substrates (Gupta and Venkitasubramanian, 1975; Lillehoj et al., 1974; Detroy et al., 1971). Several environmental, nutritional and genetic factors considerably influence the variation in the type and amount of aflatoxins produced by moulds of the genus *Aspergillus* (Davis and Diener, 1970; Lillehoj et al., 1976; Maggon et al., 1977; Zuber and Lillehoj, 1979; etc.).

The stimulatory effect of zinc on aflatoxin production is well documented (Lee et al., 1966; Maggon et al., 1973, 1977; Obidoa and Ndubuisi, 1981) and the resistance or susceptibility of natural food commodities and cultural substrates to aflatoxin formation by *A. flavus* or *A. parasiticus* has been attributed to the presence or absence of adequate amounts of trace elements especially zinc (Mateles and Adye, 1972; Lee et al., 1966; Davis et al., 1967; Bassir and Adekunle, 1972). However, phytic acid in the germ of grains strongly binds several elements including zinc (Garcia et al., 1972a,b; O'dell and Savage, 1960). The bound elements are not available biologically.

Growth of *A. flavus* and aflatoxin levels in food are related to the phytic acid level and zinc concentration available for toxin formation. Phytic acid level and zinc concentration in food are under genetic control (Zuber et al., 1978; Zuber and Lillehoj, 1979; Bassir and Adekunle, 1972). Future studies will examine the relationship between concentration of phytic acid and trace elements in other local maize varieties, and their susceptibility to aflatoxin contamination since binding of trace elements by phytates in maize germ interfere with biological availability of trace elements (Garcia et al., 1972; O'dell et al., 1972a,b).

Practical implications

Results obtained in this thesis show that the decontamination of cereal grains and grain products by the combination treatment of heat and gamma irradiation is technologically feasible. However, sanitation requirements for preventing food infection and food poisoning (by using the appropriate packaging materials) are contained in food legislations throughout the world (Elias, 1979). Articles 6, 7, 8, and 9 contain information on series of studies which compared the microbiological and physical qualities of the incumbent jute sacks used in the commercial packaging of cereal grains and legumes in Ghana with synthetic woven polypropylene sacks.

First, it has been demonstrated that grains kept in woven polypropylene sacks under practical field conditions were significantly ($P = 0.05$) more viable and had better microbiological quality than same grains stored in jute sacks (Article 6).

Secondly, there was a significant difference ($P = 0.05$) between the greater number of fungal colonies associated with jute sacks than the woven polypropylene sacks. Correspondingly, more fungal species (16) were isolated from jute sacks than from woven polypropylene sacks (9). It was also demonstrated that in the absence of exogeneous supply of nutrients 88% of the jute sack sections supported growth of fungal spores whilst woven polypropylene could not support growth of contaminating fungal spores (Article 7). Gamma irradiation (4.0 KGy) reduced the mould and yeast counts on new jute sacks and new woven polypropylene sacks by 1 and 2 log cycles respectively; but post-irradiation storage at 80% R.H. would allow growth of fungi on jute sack (Article 7). These fungi could act as springboard for infecting grain contents of jute sacks.

Thirdly, owing to the robust handling of packed cereal grains and legumes in transit, it would be ideal and desirable to use a packaging material with high tensile strength which would be able to withstand such rough handling. Saprophytic fungi on woven polypropylene could not reduce tensile strength of sections stored at 90% R.H. for 10 weeks whilst the same fungi on jute sack collectively reduced tensile strength of jute sacks by 50-75% at 90% R.H. (Article 8). Unlike jute sacks woven polypropylene sacks do not absorb moisture, they are stronger than jute sacks, do not rot under damp conditions, do not support growth of fungal spores, they are clean, do not impart any odour or taint their contents. Woven polypropylene sacks thus have many microbiological and physical advantages over the traditional jute sacks to merit their use in tropical areas like Ghana for grain storage.

Fourthly. other cereal grains (sorghum, millet) pulses (groundnut and cowpea) stored in woven polypropylene sacks were 2-3 log cycles lower in mould and yeast count than same produce kept in jute sacks for 6 months at 85% R.H. (or a_w 0.85). Significantly lower percentages of five toxigenic (*Aspergillus flavus, A. ochraceus, Fusarium moniliforme, Penicillium digitatum* and *P. expansum*) and five non-toxigenic fungi (*Aspergillus candidus, A. niger, A. terreus, A. sulphureus, A. panamensis*) were recorded on commodities stored in woven polypropylene sacks than in jute sacks (Article 9). The toxigenic and non-toxigenic fungal species collectively reduced drastically the viability of seeds kept in jute sacks for 6 months. Thus woven polypropylene sacks would also be suitable for storing other cereal grains and legumes. Woven polypropylene sacks are included in the list of packaging materials approved for irradiation by Food and Drug Administration FDA of the USA.

In the concluding experiments of this thesis, data obtained showed that the enhanced aflatoxin B_1 production by *A. flavus* spores after irradiation, the enhanced aflatoxin B_1 production by *A. flavus* spores after irradiation, reported by other workers on irradiated foods (e.g. Priyadarshini and Tulpule, 1976) could not be found on the variety of yellow maize used in the experiment. Maize varietal differences influence their susceptibility to aflatoxins contamination (Zuber and Lillehoj, 1979; Zuber et al., 1978). Future studies would examine other local maize grain varieties in relation to aflatoxin B_1 formation after the combination treatment of heat and gamma irradiation.

Twenty taste panelists in a sensory evaluation (consumer acceptance) test found no statistical difference in colour, flavour and taste between control and combined treated grains. The joint FAO/IAEA/WHO Expert Committee on Wholesomeness of Irradiated Food (Jecfi, 1980) recommended acceptability, from a toxicological standpoint of any food commodity irradiated up to an overall dose of 10 KGy. The Committee also stated that irradiated foods up to 10.0 KGy introduced no special nutritional or toxicological problems. This JECFI recommendation was adopted by the Codex Alimentarius Commission in July 1983. This established as safe for general application, irradiation up to a dose of 10 KGy. Therefore, data presented in this thesis can be applied in practice in the decontamination of maize grains (other cereal grains and legume as well). However, because irradiation does not turn a bad quality food into a healthy one, only good quality grains and legumes should be sent to irradiation facilities, packed in woven polypropylene sacks, for irradiation. Routine quarantine checks should be carried out on quality of food commodities before radiation treatment. For maximal extension of shelf-life cleanliness of the warehouse and good storage management practices should be the rule rather than the exception.

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APPENDIX

METHOD FOR DETERMINING THE INTERACTION BETWEEN ENVIRONMENTAL RELATIVE HUMIDITY AND APPLIED DOSE

Ghanaian local variety maize (*Zea mays* L) of initial moisture content (m.c.) of 13.6% was exposed (without any artificial infection) to gamma irradiation doses of 0.0, 0.8, 1.0, 2.0, 3.0, 3.5, 4.0, 4.5, and 5.0 as well as the combination treatment of moist or dry heat (60 °C for 30 min.) (Odamtten et al., 1980) prior to irradiation with 3.5 or 4.5 KGy. The Co^{60} source had a mean dose rate of 2.7 KGy h^{-1}.

Thirty grains, in each Petri dish (diameter 9.0 cm) were transferred into environmental chambers (polyethylene hood) described by Odamtten and Langerak (1980). Each chamber contained nine replicates. Potassium hydroxide solutions provided the desired humidities 70, 75, 80, 85, 90, and 95% R.H. (Solomon, 1952). The ambient humidities above the solutions were checked by leaving a Direct Reading Hygrometer (Bacharach Instrument Company) inside the environmental chambers overnight. For each radiation dose investigated, there were six humidity chambers representing all the R.H. regimes and the set up was incubated at 28 °C. Daily observations were made of the number of grains having visible external infection. From these readings, percentage infection of maize grains with time, of each treatment group was determined.

After one month, grains irradiated with 0.0-3.0 KGy and incubated at 75-95% R.H. were mouldy, mouldiness being greater at the higher R.H. regimes (80-95% R.H.).

The humidity chambers containing grains given doses up to 3.0 KGy were opened for assessment. The remaining humidity chambers containing grains exposed to 3.5-5.0 KGy were left intact for 100 days. The data after 100 days appear in Figs 1 and 2.

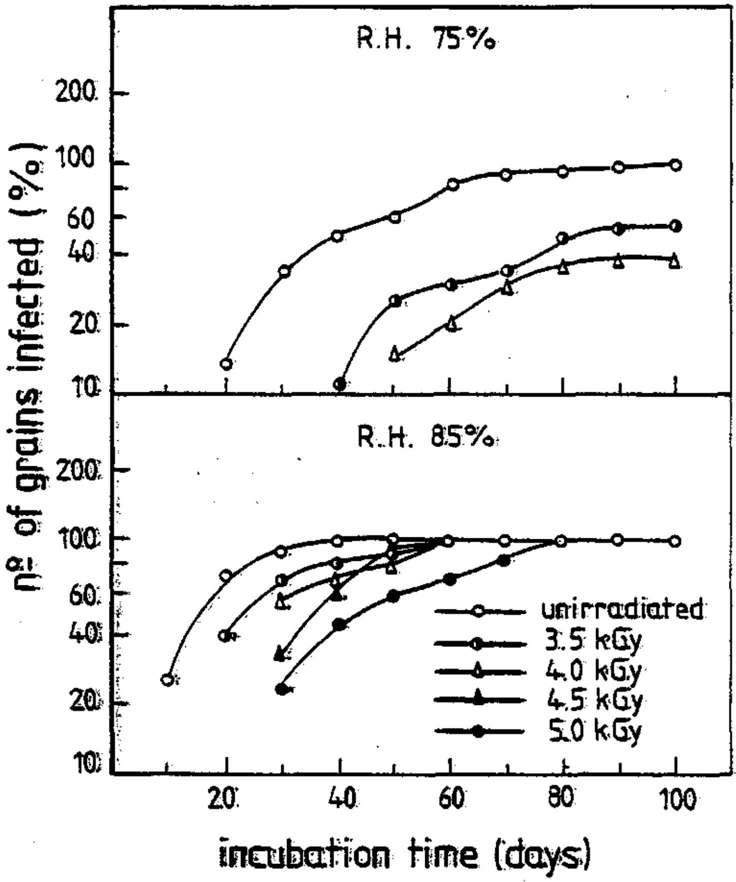

Fig. 1. Percentage spoilage of maize grains stored at 75% and 85% R.H. for 100 days after irradiation with indicated doses.

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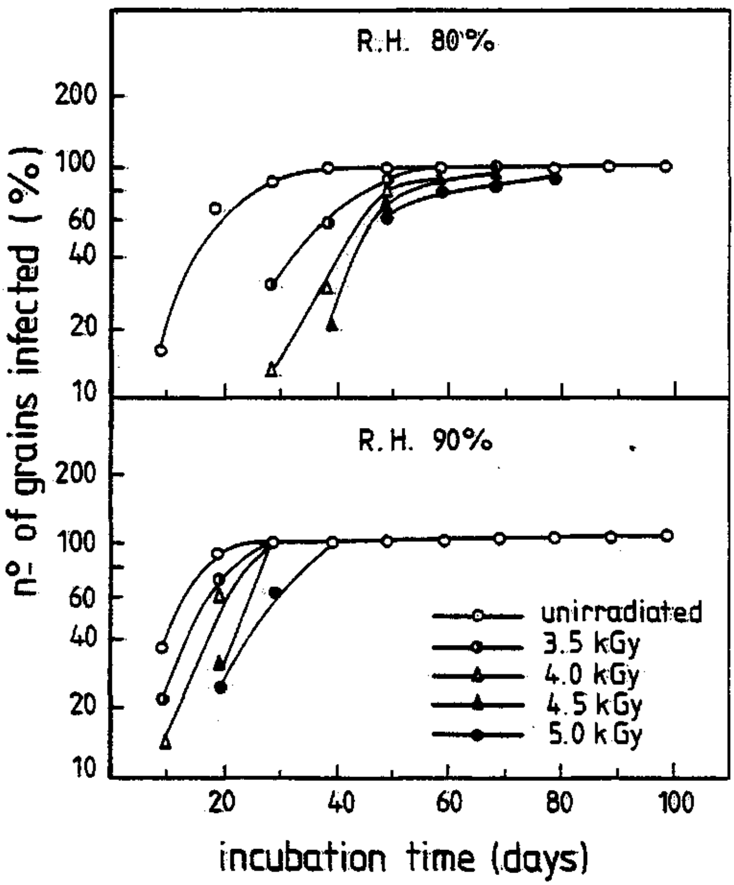

Fig. 2. Percentage spoilage of maize grains stored at 80% and 90% R.H. for 100 days after gamma irradiation with indicated doses.

13. SUMMARY

Cereal grains are important staple food crops in Africa and they are as a rule stored for long periods as buffer stock for both human consumption and lately, maize is being used as ingredient for poultry and livestock feed. The high average temperature and relative humidity (28±3 °C; >80% R.H.) permit losses in storage, due to insect activities and fungi, estimated between 30-50% of the annual harvest. Field fungi infect grains on the plant before harvest or during harvest through pure mechanical damage.
Storage fungi set in when aerial spores within the warehouse settle on bulk grains before bagging. Fungal activity discolour grains, deplete nutritive and biochemical value and reduce viability of grains. Furthermore, fungi impart potent mycotoxins into stored cereal grains. Therefore, improved quality of grains is as important as increased quantity. This thesis aims at the control of fungal deterioration of maize grains (as an example of a cereal grain) by the combination treatment of heat and gamma irradiation. The prevention of growth and production of the noxious aflatoxin B_1 formed by *Aspergillus flavus* Link was also studied. Finally, a packaging material that may extend the shelf-life of grains better than jute sack was compared with jute.

In the first article, twenty-six (26) different fungi belonging to twelve genera (*Aspergillus, Cephalosporium, Cladosporium, Curvularia, Drechslera, Fusarium, Neurospora, Paecilomyces, Penicillium, Phoma, Rhizoctonia* and *Rhizopus*) were encountered. This extended to forty-two (42) the number of seed-borne fungi recorded on maize in Ghana. Nineteen (19) of these are recorded for the first time on maize in Ghana. Members of the genus *Aspergillus* predominated followed by *Penicillium* among the species encountered. The storage fungi showed four distinct patterns of infection over the entire six months period of examination. This shows that mycoflora that would be present in any grain stock at any particular time would depend on the length of the storage period. For, indeed, *Aspergillus wentii, Cladosporium herbarum* and *Fusarium moniliforme* could not be isolated after 4 months storage. Potential toxin-producing fungi namely *A. flavus* (aflatoxins), *A. ochraceus* (ochratoxin, penicillic acid), *F. moniliforme* (moniliformin), *Paecilomyces varioti* and *Penicillium expansum* (patulin) were encountered.

In the second article, a new apparatus was designed which enabled application of either dry heat (60 °C) for 30 min) administered under low humidity (<45% R.H.) conditions or moist heat (60 °C for 30 min) administered under high (>85% R.H.) ambient relative humidity conditions. This synergistic effect lowered the gamma irradiation dose requirements form 5.0 KGy to 4.0 KGy and the treatment was more effective after exposing the spores to moist heat (60 °C for 30 min; >85% R.H.) before irradiation than when dry heat (60 °C for 30 min; <45% R.H.) was applied during the heating of the grains.

In the third article, it was demonstrated that spores of *A. flavus* NRRL 5906 irradiated in the wet state were more radiation sensitive than those treated in the dry state because while a sublethal temperature of 53 °C applied for 5 min in combination with 0.75 KGy inactivated moistered spores suspended in Tween-80 solution, a dose of 4.0 KGy was required in combination with prior moist heating (60 °C for 30 min) under 85 % R.H. to achieve the same objectives for dry spores. Fruits and vegetables which can be immersed in hot water would require much lower doses to kill contaminating spoilage fungi than dry grains infected with the same fungal spores.

In the fourth article, the nature and type of heat applied to spores of *A. flavus* was shown to have influenced vegetative growth and amount of aflatoxin B_1 formed. Aflatoxin B_1 production by *A. flavus* spores incubated in ammended maize meal broth was completely prevented by a combination of moist heat (60 °C for 30 min; >85% R.H.) applied prior to exposure of spores to 4.0 KGy of gamma irradiation but not when treated in the same way with dry heat (60 °C for 30 min; <45% R.H.) prior to irradiation. Moist heat shows greater lethality because enzymes are more readily coagulated and hydrated and, heat is transferred more readily in wet air.

The fifth article contains evidence that reduction in number of spores of *A. flavus* by 3-4 log cycles either by serial dilution, heating or its combination with irradiation led to 3-12 fold increase in aflatoxin B_1 formed by *A. flavus* spores. Thus, the aflatoxigenic potential of *A. flavus* was inversely proportional to the inoculum size: high inoculum size yielding least aflatoxin. Irradiation or its combination with heat may not have any direct effect on aflatoxin production but on the size of inoculum in relation to viable spores. Moist heat (60 °C applied for 30 min under >85% R.H.) combined with 4.0 KGy of gamma irradiation completely impaired the aflatoxigenic potential of A. *flavus* spores.

Interestingly, A. *flavus* spores failed to form aflatoxins in culture of maize meal broth alone except when the medium was autoclaved twice at 121 ^{o}C for 15 min. The excessive heating presumably release mineral elements required for aflatoxin biosynthesis.

Results in articles 2-5 have shown that decontamination of cereal grains and grain products by a combination treatment of heat and gamma irradiation is technologically feasible. However, it is imperative to prevent food re-infection by using the appropriate packaging material.

In the sixth article it was demonstrated that maize grains kept in woven polypropylene sacks under practical field conditions were significantly ($P<0.05$) more viable and had better microbiological quality than same grains stored in jute sacks.

The seventh article has data which shows that there was a significant difference ($P<0.05$) between the greater number of fungal colonies associated with jute sacks than the woven polypropylene sacks. Correspondingly, more fungal species (16) were isolated from jute sacks than from woven polypropylene sacks (9). It was also shown that in the absence of exogeneous supply whilst woven polypropylene could not support growth of contaminating spores. Gamma irradiation (4.0 KGy) reduced the mould and yeast counts on new jute sacks and new woven polypropylene sacks by 1 and 2 log cycles respectively; but post-irradiation storage at 80% R.H. allowed growth of fungi on jute sacks. These fungi on jute sacks could act as springboard for infecting grain contents of jute sacks.

The eighth article compared the tensile strength of jute and woven polypropylene sacks. Saprophytic fungi on woven polypropylene could not reduce tensile strength of sections stored at 90% R.H. for 10 weeks whilst the same fungi on jute sack collectively reduced the tensile strength of jute sacks by 50-75% at 90% R.H. after 10 weeks.

In the nineth article, it was demonstrated that woven polypropylene sacks would also be suitable for storing other cereal grains (sorghum, millet), legumes and pulses (cowpea and groundnut). These food commodities stored in woven polypropylene sacks were 2-3 log cycles lower in mould and yeast count than same produce kept in jute sack for 6 months at e.r.h. 85% (a_w 0.85). Significantly lower percentages of five toxigenic (*A. flavus, A. ochraceus, F. moniliforme, P. digitatum* and *P. expansum*) and five non-toxigenic fungi (*Aspergillus candidus, A. niger, A. sulphureus* and *A. panamensis*) were recorded on commodities stored in woven polypropylene sacks than in jute sacks. The toxigenic and non-toxigenic fungal species collectively reduced drastically the viability of seeds kept in jute sack for 6 months. Woven polypropylene sacks restricted moisture transfer to a greater extent than jute sacks reflected by the lower moisture absorbed and desorbed by commodities kept in woven polypropylene sacks than in jute sacks. Woven polypropylene thus has many microbiological and physical advantages over the traditional jute sack to merit its use in tropical areas like Ghana for grain storage.

The concluding experiments of this thesis show that enhanced aflatoxin biosynthesis by *A. flavus* spores after irradiation, reported by other workers in irradiated food could not be found in the variety of yellow maize used in the experiment. Twenty panelist in a sensory evaluation (consumer acceptance test) found no statistical difference ($P<0.05$) in colour, flavour and taste between control and combined-treated grains.

Because of the passing as safe for human consumption food irradiated with an overall dose of 10.0 KGy (JECFI, 1980), data presented in this thesis can be applied in practical decontamination of maize grains and other cereal grains and pulses as well. However, irradiation does not turn a bad quality product into a healthy one and therefore it is expedient to treat only good quality grains with combination treatment. For maximum extension of shelf-life, the recommended storage sack (woven polypropylene) should be used in bagging prior to irradiation. Cleanliness of the warehouse and good storage management practice should be rigidly adhered to.

14. SAMENVATTING

Granen zijn een belangrijk volksvoedsel in Afrika. Deze worden gewoonlijk voor langere tijd opgeslagen als voorraad voor menselijke, en in toenemende mate ook voor dierlijke consumptie. Bij hoge gemiddelde temperatuur en relatieve vochtigheid (28±3 °C ; >80% R.V.) worden bewaarverliezen veroorzaakt als gevolg van ontwikkeling van insecten en schimmels. Deze bewaarverliezen worden op 30-50% van de oogst geschat. Besmetting met schimmels treedt op vóór en tijdens de oogst, maar ook in het pakhuis, vóór het afvullen. Schimmelgroei kan resulteren in verkleuring van korrels, verlies van nutriënten en verlies van kiemkracht. Ook kunnen mycotoxinen worden gevormd.

De doelstelling van het onderzoek beschreven in dit proefschrift is het beheersen van schimmelbederf van maïs door gecombineerde behandeling met hitte en gammastralen. De remming van de groei en aflatoxine B_1 produktie door *Aspergillus flavus* Link werd tevens bestudeerd. Tenslotte werd de houdbaarheid van granen in een nieuw verpakkingsmateriaal vergeleken met die in de gangbare jutezakken.

In het eerste artikel worden uit maïs 26 schimmelsoorten geïsoleerd, behorend tot de volgende 12 geslachten: *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phoma*, *Rhizoctonia* en *Rhizopus*. Het betreft overwegend soorten van *Aspergillus* en voorts van *Penicillium*. Het bleek dat de mycoflora die op een bepaald tijdstip in een graanvoorraad wordt aangetroffen, afhangt van de bewaarduur. Zo konden *Aspergillus wentii*, *Cladosporium herbarum* en *Fusarium moniliforme* na vier maanden bewaarduur niet meer geïsoleerd worden. Potentiële toxinevormers, die werden aangetroffen, waren: *A. flavus* (aflatoxine), *A. ochraceus* (ochratoxine, penicillinezuur), *F. moniliforme* (moniliformine), *Paecilomyces varioti* en *P. expansum* (patuline).

Het tweede artikel beschrijft de ontwikkeling van een apparaat waarmee hittebehandelingen (60 °C , 30 min.) bij lage (<45%) en hoge (>85%) relatieve luchtvochtigheid kunnen worden uitgevoerd. Door deze behandeling kon de, voor afdoende schimmeldoding benodigde dosis gammabestraling van 5.0 kGy tot 4.0 kGy verlaagd worden. Daarbij was de vochtige warmtebehandeling, uitgevoerd vóór de bestraling, effectiever dan de droge warmtebehandeling.

In het derde artikel wordt aangetoond dat sporen van *A. flavus* NRRL 5906 in natte toestand bestralingsgevoeliger zijn dan in droge vorm. Sporen gesuspendeerd in een Tween 80 oplossing werden gedood na een behandeling van 5 min. bij de sublethale temperatuur van 53 °C, in combinatie met een bestralingsdosis van 0.75 kGy, terwijl 4.0 kGy nodig was voor droge sporen die een vochtige warmtebehandeling hadden ondergaan (30 min bij 60 °C, relatieve vochtigheid >85%). Groenten en fruit, waarvoor een warmwaterbehandeling mogelijk is, zouden daarom met een veel lagere bestralingsdosis behandeld kunnen worden dan droge granen.

In het vierde artikel wordt aangetoond dat de aard van de hittebehandeling invloed heeft op de vegetatieve groei en daarmee op de produktie van aflatoxine B_1. De vorming van dit toxine in een verrijkt maïsmeel medium kon worden voorkomen met behulp van een behandeling met vochtige warmte (30 min 60 °C, >85% relatieve vochtigheid) gevolgd oor gammabestraling met 4.0 kGy, maar niet door een combinatiebehandeling met droge warmte (30 min 60 °C, <45% relatieve vochtigheid).

Het vijfde artikel bevestigt gegevens uit de literatuur dat een reductie van de concentratie van sporen van *A. flavus* met drie tot vier logcycli òf door verdunning, òf door hittebehandeling òf door hitte gecombineerd met bestraling leidt tot een drie- to twaalfvoudige toename in aflatoxine B_1 produktie. Het vermogen om aflatoxine te produceren was dus omgekeerd evenredig met de omvang van het inoculum. Bestraling, of de combinatie daarvan met een hittebehandeling heeft dan ook wellicht geen direct effect op aflatoxine-produktie, maar slechts op het oorspronkelijke aantal levende sporen. Na een gecombineerde behandeling van *A. flavus* sporen met vochtige warmte (30 min 60 °C, relatieve vochtigheid >85%) en 4.0 kGy gammabestraling kon geen aflatoxinevorming meer aangetoond worden.

Het is interessant dat *A. flavus* geen aflatoxine vormt in een culture van uitsluitend maïsmeel medium, maar wel indien dit medium twee keer 15 min was geautoclaveerd bij 121 °C. Door deze hittebehandeling worden mogelijk mineralen vrijgemaakt, die nodig zijn voor de biosynthese van aflatoxine.

In de artikelen twee tot en met vijf wordt aangetoond dat decontaminatie van granen door een gecombineerde behandeling van hitte en bestraling technologisch uitvoerbaar is. Daarbij is echter het voorkómen van herbesmetting door het gebruik van geschikt verpakkingsmateriaal noodzakelijk.

In het zesde artikel wordt aangetoond dat maïs bewaard in geweven polypropyleen zakken, onder praktijkomstandigheden significant ($P = 0.05$) kiemkrachtiger bleef en microbiologisch bezien zijn kwaliteit beter behield dan maïs in jutezakken.

Uit gegevens in het zevende artikel blijkt dat het schimmel-kiemgetal van jutezakken significant hoger is ($P < 0.05$) dan dat van polypropyleen zakken. Er werden ook dienovereenkomstig meer schimmelsoorten (16) geïsoleerd van jutezakken dan van polypropyleen zakken (9). Ook bleek dat, indien geen andere substraten aanwezig waren, in 88% van de monsters van jutezakken de ontwikkeling van schimmelsporen mogelijk was, terwijl bij polypropyleen de groei van schimmels niet mogelijk was. Bestraling (4.0 kGy) reduceerde de kiemgetallen van schimmels en gisten op nieuwe jutezakken en op nieuwe geweven polypropyleen zakken, respectievelijk met een en twee logcycli. Maar bij bewaring bij 80% relatieve vochtigheid na bestraling trad bij jutezakken schimmelgroei op. Deze schimmels op jutezakken zouden infectie van de inhoud kunnen veroorzaken.

In het achtste artikel wordt de treksterkte van jute- en geweven polypropyleen zakken vergeleken. Saprofytische schimmels konden de treksterkte van monsters van polypropyleen zakken, tien weken bewaard bij 90% relatieve vochtigheid, niet doen afnemen, terwijl dat wel het geval was bij jutezakken (50-75% verlies van treksterkte).

In het negende artikel wordt aangetoond dat geweven polypropyleen zakken ook geschikt zijn voor de opslag van andere granen (sorghum, millets) en peulvruchten ('cowpea' en aardnoten). Kiemgetallen (gisten en schimmels) van deze produkten waren, na 6 maanden bewaring bij 85% rel. vochtigheid, twee tot drie logcycli lager in polypropyleen zakken dan in jutezakken. Daarbij werden tevens beduidend lagere percentages toxigene (*A. flavus*, *A. ochraceus*, *F. moniliforme*, *P. digitatum* en *P. expansum*) en niet-toxigene schimmels (*Aspergillus candidus*, *A. niger*, *A. sulphureus* en *A. panamensis*) gevonden in produkten, bewaard in polypropyleen zakken. De toxigene en niet-toxigene schimmels veroorzaakten een aanzienlijke verlaging van de kiemkracht van zaden na zes maanden bewaring in jutezakken. Geweven polypropyleen zakken zijn minder vochtdoorlatend dan jutezakken. Dit resulteert in geringere vochtabsorptie en -desorptie door produkten in polypropyleen zakken. Geweven polypropyleen zakken hebben dus microbiologische en technische voordelen boven de traditionele jutezakken en verdienen aanbeveling in tropische landen als Ghana.

In een afrondend onderzoek bleek een verhoogde aflatoxine biosynthese door *A. flavus* na bestraling, zoals in de literatuur vermeld, niet aangetoond te kunnen worden bij het gele maïsras dat daarvoor werd gebruikt. In een sensorische evaluatie, waarbij met 20 panelleden een "consumer acceptance test" werd uitgevoerd, werd geen significant verschil ($P < 0.05$) in kleur, geur en smaak waargenomen tussen maïs waarop de gecombineerde behandeling was uitgevoerd en de blanco.

CURRICULUM VITAE

George T. Odamtten was born on 7th July 1948 at Koforidua in the Eastern Region of Ghana. After his Primary School education at the Presbyterian Primary Schools at Suhum and Accra (1952-1958), he proceeded to the Presbyterian Boys Boarding School, Osu, Accra from where he entered the Accra Academy for his Secondary School education (1962-1969). He obtained the West African Examination Council's General Certificate in Education (G.C.E., Ordinary Level) in 1967 and the University of London Advanced Level Certificate in 1969.

He had his University education at the University of Ghana, Legon, from October 1969 to 1974 during which he obtained the B.Sc. General degree (Botany, Zoology) in 1973 and the B.Sc. Honours Botany degree (Microbiology and Plant Pathology) in 1974. From September 1974 to August 1975 he was a Teaching Assistant in the Department of Botany where he assisted in both research and teaching. He was awarded an M.Sc. Botany degree in Mycology by the University of Ghana in 1977.

Later, in September 1977, he joined the staff of the Ghana Atomic Energy Commission, Department of Biology, Food and Agriculture, as a Scientific Officer (Microbiology). His applied research interests were post-harvest pathology as well as biological control of plant pathogens. He was awarded an applied research fellowship by the International Atomic Energy Agency to participate in the course of study at the 1st IFFIT Interregional Training Course on Food Irradiation (Wageningen, The Netherlands, 4th Sept. 1979 - 12th Oct. 1979), during which he obtained a Certificate in Food Irradiation Technology. Thereafter, he continued his applied research in the control of microbial spoilage of cereals, cocoa, animal feed, cotton seeds and aubergines employing a new technique of combination treatment of heat and gamma irradiation.

This work forming the thesis was carried out at ITAL, Wageningen and then continued on his return home at the University of Ghana. He lectured in Botany on part-time basis at the University of Ghana, Legon from 1981 to February 1982 and then became full-time Lecturer in March 1982 whilst he continued his research work under the supervision of Prof.Dr. E.H. Kampelmacher, during the period 1981-1985. The present address of the candidate is Department of Botany, University of Ghana, Post Office Box 55, Legon/Accra, Ghana.

Aangezien bestraling van voedingsmiddelen met een totale dosis van max. 10 kGy als veilig wordt geaccepteerd (JECFI, 1980), kon de in dit proefschrift voorgestelde behandeling voor ontsmetting van maïs en andere granen en peulvruchten in de praktijk worden toegepast. Bestraling verandert een produkt van slechte kwaliteit echter niet in een goed produkt, en daarom is het zaak deze behandeling alleen op grondstoffen van goede kwaliteit toe te passen. Voor een maximale houdbaarheid moet het produkt in de aanbevolen emballage (geweven polypropyleen zakken) verpakt worden vóór de bestraling. Daarbij dient men zich strikt te houden aan een hygiënische bedrijfsvoering in het pakhuis.