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Full Length Research Paper

Fungal and bacterial contaminants of six spices and spice products in Ghana

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The microbiological quality of two spices (aniseed and rosemary) and spice products (maggi onion cube, maggi shrimp cube, royco shrimp cube, royco beef cube) have been studied using conventional mycological techniques. The presence of moulds and yeast was compared on three media DRBC, OGYE, and PDA at 30°C incubated for 5 days. The species of fungi that were isolated from the raw spices and spice products tested belonged to eight (8) genera. *(Absidia, Aspergillus, Cladosporium, Fusarium, Rhizopus, Penicillium, Neurospora, Eurotium,). Aspergillus species (A. flavus, A. fumigatus, A. alutaceus, A. niger, A. sulphureus*) were the more predominant species isolated. *A. flavus* was the most frequently isolated fungal species in all the spices on all the three media on which growth was compared. Aniseed harboured fungal population ranging from 1.50 $\log_{10}$ CFU/g sample to 1.88 $\log_{10}$ sample; maggi onion cube 0.90 – 1.54 $\log_{10}$ CFU/g sample; maggi shrimp cube 1.11 – 1.30 $\log_{10}$ CFU/g sample; royco shrimp cube; 1.0 – 1.08 $\log_{10}$ CFU/g sample and 1.19 – 1.31 $\log_{10}$ CFU/g sample in royco beef cube. The aflatoxin analysis showed that aflatoxins B_1, B_2, G_1, and G_2 were not formed. The bacteria species isolated from the spices varied. The spices and spice products collectively harboured *Aeromonas salmonicida, Enterobacter cloacae, Enterobacter amnigenus, Enterobacter agglomerans, Enterobacter sakazakii, Flavobacterium* sp*, Chromobacterium violaceum, Pseudomonas putida, Pseudomonas aeroginosa, Acinetobacter* sp*, Pseudomonas cepacia, Serratia plymuthica.* The human health implications of these findings are discussed and future work recommended.

Key words: Microbiological quality, spices, spice products, contaminants.

INTRODUCTION

The impact of media advertisement on the use of various spices and herbs to flavour liquors and the medicinal properties of spices such as aniseed (*Pimpinella anisum*) and rosemary (*Rosemarinus officinalis*), has deemed it necessary to evaluate the microbiological quality of these spices and herbs largely patronized by Ghanaians. Aniseed *(P. anisum*) is a spice belonging to the family apiaceae. The fruits which are often termed 'seeds' are used in the dried form; it is sweet and aromatic. The aroma of the essential oil (up to 3% in the fruit) is dominated by trans-anethole (max 90%) (Gernot, 1998).The main application of this spice is in flavouring liquors, used to aromatize fruit products and for culinary use. It also works as a tonic and as an expectorant.

Rosemary (*R. officinalis*) belongs to the family Lamiaceae. The plant part used is the small needle-like leaves. It is used fresh, dried or in the powdery form. It is strongly aromatic (reminiscent of camphor or eucalyptus) resinous and slightly bitter. The leaves contain about 1 to 2% essential oil, therein, 1, 8-cineol (30%), camphor (15 to 25%), borneol (16 to 20%), bornyl acetate (max 7%) α-pinene (max 25%). Rosemary is used for culinary purposes and in cosmetics. Studies carried out in the last several years showed that oil from the leaves of Rosemary can help prevent the development of cancerous tumours in laboratory animals (Anon, 2006). One study showed that applying rosemary oil to the skin of experimental animals reduced their risk of cancer to half of that found in animals that did not receive the application of the oil. Animals whose diets contained

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rosemary oil had about half the incidence of colon cancer or lung cancer compared with animals not eating rosemary (Gardenguides, 2002). Rosemary cut by half, the incidence of breast cancer in animals at high risk for developing the disease. The oil is not taken orally. It has other medicinal properties such as serving as a muscle relaxant of the smooth muscles of the digestive tract and uterus. Thus it soothes digestive upsets and relieves menstrual cramps. It also makes a pleasant tasty tea with a teaspoon of crushed dried leaves in a cup of boiling water (Gardenguides, 2002). Rosemary is also used as an insect repellent (kills cabbage moths, bean beetles and carrot flies).On the Ghanaian market, there are numerous mixtures of spices that are well patronised. Some of these are maggi products (for example maggi onion, maggi shrimp cube) from Nestlé Ghana limited and royco products (for example royco beef, royco shrimp cube) from Unilever Ghana, limited.

Maggi onion contains iodised salt, flavour enhancers, starch, vegetable fat, sugar, aniseed, cloves, onion and other spices while Maggi shrimp contains iodised salt, flavour enhancers, starch, vegetable fat, sugar, shrimp powder and spices (not specified). As a trade secret, the exact compositions of the ingredients are not known to the consumer.

Spices and herbs may be contaminated because of the conditions under which they were cultivated and harvested. Contaminated spices have been reported to have been the causes of certain food-borne illnesses and spoilage (Giese, 1994).

The microbiological contamination of spices may arise from sources, such as indigenous microflora of plants, microorganisms present in processing plant, air, post harvest contamination from dust, use of contaminated water and from human contact, (Wirtanen and Sjoberg, 1993). During cleaning and processing, there is progressive reduction in the number and types of microorganisms; those remaining are usually aerobic spore-forming bacteria and common moulds (Guarino, 1973). In addition to the contamination of raw food supplies that occurs during growing, shipping and processing, there is the problem of food contamination caused by people who are carriers of pathogens such as *Salmonella, Escherichia coli* and *Staphylococcus aureus*. Even though few food borne outbreaks have been traced to the consumption of contaminated spices, numerous isolations of pathogens, from a variety of spices, including oregano, black pepper and white pepper (whole and in powder form) have been reported (Wilson and Andrew, 1976).

In costa Rica, the microbiological quality of some of the powdered spices usually used in homes, without further thermal treatment was evaluated. Analysis of black, white and green peppers showed that the total plate counts and faecal coliforms exceeded the international commission on microbiological specifications for foods (ICMSF) standards and that contamination was probably due to drying conditions and post-harvest treatment (Arce, 1990). A total of 75 samples of five different powdered spices (onion, garlic, oregano, pepper) randomly acquired in supermarkets in the Metropolitan area of San Jose were analysed in the laboratory. It was observed that there was considerable variations in the total aerobic plate count ranging from $\leq$ 100 CFU/g to more than 1×10^7CFU/g. Garlic, probably due to the marked bactericidal effect of its essential oil, had the lowest percentage of samples of the ICMSF proposed norm (26.7%), followed by pepper (37.5%), black pepper (64.3%) and oregano (73.3%). The low count in garlic samples may probably be attributed to the marked bactericidal effect of its essential oil (Frazier and Westohoff, 1978).

Caulley (1990), and Paintsil (1996), examined mycoflora in stored fresh ginger and powdered ginger. They recorded the occurrence of *Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus ochraceus, Aspergillus glaucus, Aspergillus niger, Cladosporium herbarum, Fusarium, Penicillium digitatum,* and *Trichoderma viride*. These fungal species showed five patterns of infection. *A.niger, A. fumigatus*, and *A. nidulans* showed a continuous decline in population during the three months storage period; *A. ochraceus* and *P. digitatum* increased in population during the same period while others for example *A. glaucus, A. terreus, P. expansum* and *Fusarium* species reach peak population after one month of storage and thereafter declined. There was sporadic occurrence of *C. herbarum* and *T. viride* after three months while population of *A. flavus* declined during the first month of storage and thereafter increased to the same level as at the onset of the experiment.

Addo (2005), tested the microbiological and nutrient quality of three local spices (ginger, mixed ginger and garlic and mixed spices) and showed that ginger harboured a fungal population ranging from 3.08$\log_{10}$ CFU/g sample potato dextrose agar (PDA) to 2.40 $\log_{10}$ CFU sample on DG18. The total aerobic bacteria counts in the spices ranged from 3.6 $\log_{10}$CFU/g sample (in ginger) to 3.7 $\log_{10}$ CFU/g sample (in mixture of ginger and garlic) bacterial species which collectively contaminated the spices were *Aeromonas salmonicida, Enterobacter cloacae, Enterobacter sakazakii, Pasteurella* sp*., Proteus* sp*., Pseudomonas* sp*., Salmonella* sp*., Flavobacterium meningosepticum, Sphigom*onas *paucimobilis* and *Xanthomonas maltophilia (*Addo, 2005). There are therefore serious health implications in the consumption of such spices on the market (Addo, 2005).

Flannin and Hui (1996) also showed that moulds associated with spices and herbs were predominantly *Aspergillus* sp. of which seven (7) out of twenty-four (24) strains produce aflatoxins *in vitro.*

These fungal species impart toxic metabolites

(mycotoxins) during growth in food and food products making food unfit for human consumption. The mycotoxins with the most potential human health hazards are toxins of storage fungi in the genera *Aspergillus, Penicillum* and *Fusarium*. The most potent are aflatoxins B_1, B_2, G_1 and G_2 (produced by *Aspergillus flavus, A. nomius* and *A. parasiticus*), Fumonisins and trichothecenes (produced by *Fusarium* sp) and patulin (produced by *Penicillium* sp) (Richard, 2000).

Royco and Maggi cubes are spices manufactured in Ghana; they are popular and have a nationwide patronage. Their formulation although not known for certain by the public includes some spices such as rosemary and aniseed.

This paper reports of findings of a research carried out to assess the microbiological quality of two spices and four spice products on the Ghanaian market namely, royco shrimp, royco beef, maggi onion and maggi shrimp. It also provides information on the aflatoxigenic potential of the *A. flavus* isolated from the spices and spice products.

MATERIALS AND METHODS

Source of spice samples

The six spices and spice products used in this study were obtained from the local market (Makola market). These were aniseed (*P. anisum*), rosemary (*R. officinalis*), royco shrimp, royco beef, maggi onion and maggi shrimp cubes. Aniseed and rosemary were packaged in transparent polyethylene bags.The spice products (royco shrimp, royco beef, maggi onion and maggi shrimp) were packaged in paper with an aluminium foil draped in it with each cube weighing 10 g except royco beef which weighed 4 g. They were placed in resealable polyethylene bags and transported to the laboratory.

Microbiological analysis

Quantitative estimation of mycoflora population

The initial mycoflora in the spices was determined by transferring 10 g samples into 250 ml Erlenmeyer flasks containing 100 ml of 0.1% peptone water as diluents. Each flask was shaken at 140 RPM for 20 minutes on an orbital shaker (Gallenkamp, England). Serial dilutions up to $1:10^4$ were made and 1 ml aliquots were plated on 20 ml of oxytetracycline glucose yeast extract agar (OGYE) Oxoid ,CM 545), dichloran rose bengal chloramphenicol agar (DRBC), Oxoid CM 727) and PCA (plate count agar, BIOCHEMIKA 70152). All the plates were incubated at 30 °C for 5 days. Colonies of mycoflora that appeared after 5 days of incubation were counted and calculated as $\log_{10}$ CFU/g sample.

Quantitative estimation of total aerobic bacteria

The initial bacteria population in the spices was determined by transferring 10 g samples into 250 ml Erlenmeyer flasks containing 100 ml of 0.1% peptone water as diluents. Each flask was shaken at 140 RPM for 20 min on an orbital shaker (Gallenkamp, England). Serial dilutions up to $1:10^4$ were made and 1 ml aliquots were plated on 20 ml PCA (plate count agar, BIOCHEMIKA 70152). They were incubated at 35°C for 48 h. Colonies of bacteria that appeared were counted and calculated as $\log_{10}$ CFU/g sample.

Isolation and identification of bacteria

Bacteria colonies that developed on PCA were sub-cultured on nutrient agar until a pure culture was obtained. These pure cultures were used for identification using an analytical profile index (API) 20E kit (bioMérieux). Reactions were read according to a standard reading table and identification was done using the analytical profile index book.

Aflatoxigenic potential of *Aspergillus flavus* isolated from the spices

Thirty millilitres of spice extracts of the spices (aniseed, rosemary, maggi onion cube, maggi shrimp cube, royco shrimp cube, and royco beef cube) prepared above in triplicates were inoculated with 1 ml spore suspension of *A. flavus* (about 1.0×10^4 cfu/g). The Erlenmeyer flasks were incubated for 5 days at 30°C. Growth in the liquid medium was assessed by estimating the dry weight of the harvested mycelium. Mycelium collected on a previously weighed and dried Fischer Brand filter paper was dried at 75°C for 24 h and then reweighed after cooling in a desiccator. The culture filtrates were retained for pH determination and extraction of aflatoxin.

Determination of Aflatoxin levels in the spices

This was determined using the high performance liquid chromatography (HPLC) Equipment at the Food Research Institute, CSIR-Ghana, following the method outlined by Pons (1979). The culture filtrate of *A. flavus* from each of the spices was subjected to aflatoxin determination.

pH measurement

This was carried out using a TOA pH meter (Model, HM- 60S) from Ogawa Seiki Company. Limited, Japan.

Statistical analysis

Statgraphics plus (windows 3.0) and Duncan's multiple test. Results were quoted at $p \leq 0.05$ level of significance.

RESULTS

Microbiological quality of spices and spice product

The population of fungi that was resident in the spice and spice products was dependent on the type of medium used to isolate the species. Generally, aniseed harboured fungal population ranging from 1.50 $\log_{10}$ CFU/g sample on PDA and 1.88 $\log_{10}$ CFU/g sample on DRBC. The level of mycoflora was slightly higher on DRBC than on OGYE agar and PDA for aniseed, rosemary and maggi shrimp cube by less than 1.0 log cycle (Table 1).

However, comparatively on the same media, the

Table 1. Microbiological quality of the indicated spices and spice products plated on PDA, OGYE or DRBC (for fungi) and PCA (for bacteria).

Types of spice	Total microbial population (log_{10}CFU/g) on			
	PDA	OGYE	DRBC	PCA
Aniseed	1.50	1.72	1.88	3.63
Rosemary	1.35	1.36	1.63	3.62
Maggi onion cube	1.54	0.60	0.90	3.30
maggi shrimp cube	1.11	1.20	1.30	3.06
royco shrimp cube	1.01	1.08	1.00	3.55
Royco beef cube	1.31	1.80	1.19	3.59

Table 2. List of fungal species isolated from raw spices and spice products on the Ghanaian market plated on OGYE and DRBC at 30°C for 5 days.

Fungal species on OGYE	Aniseed	Rosemary	MOC	MSC	RSC	RBC
Absidia ramosa	_	+	_	_	_	_
A. alutaceus	+	_	+	_	+	+
A. flavus	+	+	_	+	+	+
A. fumigatus	_	_	_	+	+	+
A. niger	_	_	_	+	+	+
A. sulphureus	+	+	_	_	_	_
Cladosporium macrocarpum	+	_	_	_	_	_
Erotium amstelodami	+	_	_	_	+	+
Neurospora sitophila	_	+	_	_	_	_
Penicillium sp.	+	_	+	_	_	+
Rhizopus stolonifer	_	+	_	_	_	_
Fungal species on DRBC						
A. alutaceus	+	+	_	+	_	+
A. flavus	+	+	+	+	+	+
A. fumigatus	+	+	_	+	+	+
A. niger	+	+	_	_	+	_
Caldosporium macrocarpum	+	_	_	_	_	_
Fusarium verticilloides	+	_	+	+	+	+
Penicillium sp	_	+	_	_	_	_
Rhizopus stolonifer	_	+	_	_	_	_

+ Present, - Absent

difference in population in the six raw spices and spice products was between 0.21 – 0.54 log cycles on PDA, 0.48 – 1.20 log cycles on OGYE and 0.10 – 0.98 log cycles on DRBC. Total aerobic bacteria varied from 3.06 log_{10} CFU/g sample (in maggi shrimp) to 3.63 log_{10} CFU/g sample (in aniseed) (Table 1). Generally, the difference in bacterial population was less than 1.0 log cycle. In all the three media used for fungal growth, the occurrence of *Aspergillus* species in the raw spice and spice products predominated over the other fungi encountered. These were *A. alutaceus, A. flavus, A.fumigatus, A. niger* and *A. sulphureus*. One *Penicillum* sp and *Fusarium* sp were encountered (Table 2). The species of fungi encountered in the raw spices and products varied from one sample to another.

Even though the population of resident mycoflora was low (1.50-1.8 log_{10} cfu/g sample), there were other fungal species of pathological importance found in all the samples. Using two different media (DRBC and OGYE) gave a wider spectrum of fungi. *Aspergillus* sp *(A. alutaceus, A. flavus, A. fumigatus, A. sulphureus, A. niger*) of pathological importance were found (Table 2).

A. flavus (a fungus with the ability to produce aflatoxins) was the most frequently isolated. Other

Table 3. List of bacterial species isolated from raw spices abd spice productsn the Ghanaian market plated on PCA at 35 to 37°C FOR 48 h.

Bacterial species	Aniseed	Rosemary	MOC	MSC	RSC	RBC
Acinetobacter sp	_	_	+	_	+	_
Aeromonas salmonicida	+	+	+	+	+	+
Chromobacterium violaecum	+	_	_	_	_	_
Chryseomonas luteola	+	_	+	+	_	_
Enterobacter cloacae	+	+	_	+	_	_
Enterobacter sakazakii	+	+	_	+	+	+
Enterobacter agglomerans 1	+	+	+	+	_	_
Enterobacter anmigenus 2	+	+	_	+	_	_
Flavobacterium sp	+	+	_	_	_	_
Pseudomonas cepacia	_	_	+	_	+	+
Pseudomonas fluorescens	+	+	_	_	_	_
Pseudomonas putida	+	+	_	_	_	_
Pseudomonas aeroginosa	+	+	_	_	_	_
Pseudomonas sp	+	_	_	_	_	_
Serratia plymuthica	_	_	+	+	_	+

+ Present, - Absent

Table 4. Vegetative growth of *Aspergillus flavus* in 40 g/l extract of the indicated spices at 30 °C for 5 days.

Type of spices	pH of medium		Dry weight of mycelium(mg)
	Initial	Final	(Mean ±S.E.)
Aniseed	5.58	5.92	43.33± 5.77
Rosemary	5.71	8.06	153.33±20.82
Maggi onion cube	6.11	5.97	216.67± 5.77
Maggi shrimp cube	7.80	7.53	176.67±15.27
Royco shrimp cube	8.11	8.41	136.67±11.54
Royco beef cube	7.26	7.60	96.67± 5.77

mycotoxigenic fungi such as *A. alutaceus* (ochratoxin), *Fusarium verticilloides* (fumonisins and moniliformin) were also isolated (Table 2).The results for bacterial load of the spices and spice products are presented in Table 3. Enteric bacteria viz *Enterobacter amnigenus 2*, *E. agglomerans 1*, *E. cloacae, E. sakazakii* and other gram negative bacteria viz *Acinobacter* sp. *Aeromonas salmonicida, Chryseomonas luteola, Flavobacterium* sp. *Chromobacterium violaceum, Pseudomonas aeroginosa, P. putida and P. fluorescens were encountered*. Aniseed harboured the highest number of bacterial load, while the least was encountered on royco shrimp cube and royco beef cube.

Influence of the raw spices and spice products on vegetative growth and aflatoxin production by *Aspergillus flavus* in liquid media The influence of aqueous extracts of the raw spice and spice products (royco and maggi cubes) on the growth of *A. flavus* was studied *in vitro*. The various spice extracts supported good vegetative growth of *A. flavus* (Table 4). However, rather curiously, analysis for aflatoxins did not show the presence of aflatoxins B_1, B_2 G_1, and G_2 (Table 5). The spices variably supported vegetative growth of *A. flavus in vitro* (Table 4). The best growth was obtained from maggi onion cubes (216.67 mg) followed by maggi shrimp cubes (176.67 mg); the least growth was observed for aniseed spice (43.33 mg). Clearly all the spices supported appreciable growth of *A. flavus.* The pH of the media drifted only slightly during the 5 days growth in culture (Table 4).

DISCUSSION

The survival of fungal species on dehydrated products is well known. The use of available microbiological techniques to determine the quality of spice products for human consumption is the rule rather than the exception.

Though the population of resident mycoflora was low (1.50-1.8 $\log_{10}$ cfu/g sample), there were other fungal

Table 5. Aflatoxin analysis of spice samples with the indicated codes after growth of *Aspergillus flavus* for 5 days at 30°C using HPLC technique.

Sample code	Aflatoxins(µg/kg)				Total
	B_1	B_2	G_1	G_2	
RB (FRI MTR:06/88)	ND	ND	ND	ND	ND
RS (FRI MTR:06/89)	ND	ND	ND	ND	ND
MS (FRI MTR:06/90)	ND	ND	ND	ND	ND
MO (FRI MTR:06/91)	ND	ND	ND	ND	ND
ROSE (FRI MTR:06/92)	ND	ND	ND	ND	ND
ANI (FRI MTR:06/93)	ND	ND	ND	ND	ND

Detection limit: Aflatoxin B_1 andB_2 = 0.04 µg/kg, Aflatoxin $G_1$and G_2 = 0.06 µg/kg, RB – royco beef cube, RS – royco shrimp cube, MO – Maggi Onion cube, ANI- aniseed, ROSE – rosemary, MS – maggi shrimp cube, ND – not detected.

species of pathological importance resident in all the samples. Using two different media (DRBC and OGYE) gave a wider spectrum of fungi. *Aspergillus* sp *(A. alutaceus, A. flavus, A. fumigatus, A. sulphureus, A. niger*) of pathological importance were encountered in the samples tested. *A. flavus* (a fungus with the ability to produce aflatoxins) was the most frequently isolated *Aspergillus* species in all the spice samples. Other mycotoxigenic fungi for example. *A. alutaceus* (ochratoxin), *Fusarium verticilloides* (fumonisins and moniliformin) were also isolated. The isolation of these mycotoxigenic fungi agrees with work done by Paintsil, (1996) and Addo, (2005). The health implications of consuming such contaminated spices are enormous especially in Africa where there is correlation between Hepatitis B and aflatoxins. Prevalence of hepatitis B markers was estimated from the serum of blood donors, and liver cancer incidence was recorded for the years 1979 to 1983 through a national system of cancer registration. Across 4 broad geographic regions, there was a more than 5-fold variation in the estimated daily intake of aflatoxin, ranging from 3.1 to 17.5 µg. The proportion of hepatitis B virus (HBV)-exposed individuals was very high (86% in men), but varied relatively little by geographic region; the prevalence of carriers of the surface antigen was 23% in men, and varied from 21 to 28%. Liver cancer incidence varied over a 5-fold range, and was strongly associated with estimated levels of aflatoxin (Peers et al., 1987).

Bacterial species, including five members of Enterobacteriaceae, *Enterobacter agglomerans, E. cloacae,* and *E. sakazakii* were isolated in (aniseed and rosemary), *Serratia plymuthica* (in maggie onion cube and maggie shrimp cube) whereas *Aeromonas salmonicida* and *Pseudomonas* sp. were encountered in all the samples. The other species of bacteria were *Flavobacterium* (aniseed and rosemary), *Chromobacterium violaceum* (aniseed), *Pseudomonas putila* (aniseed and rosemary), *P. aeroginosa* (aniseed), *Acinobacter* sp. (royco shrimp and maggie onion) and *P. cepacia* (maggie onion, royco shrimp and royco beef). Previous studies by (Addo, (2005) have shown that similar microorganisms contaminate Ghanaian spices. Bacterial species, belonging to the family Enterobacteriaceae frequently causes diarrhoea and gastrointestinal infection accounting for an annual mortality rate of five million people worldwide (Brooks et al., 2001) It is the second most common cause of death after cardiovascular illness (Talaro and Talaro, 1993). The preponderance of mycotoxigenic *Aspergillus* species as well as Enterobacteriaceae in the spice samples presumable predisposes consumers to mycotoxins contamination and diarrhoea and other opportunistic diseases. This is not desirable for product stability and safety for human consumption.

Undoubtedly, aflatoxins produced by *A. flavus, A. parasiticus* and *A. nomius* in various commodities including maize, rice, barley, wheat, sorghum, groundnut and oil foods, copra and presumably in some spices have attracted the attention of international health authorities, (CDC, 2004). Aflatoxins are immunosuppressive, mutagenic, teratogenic and carcinogenic in their effect with the main target organ being the liver. Evaluation of epidemiological and laboratory results carried out in 1987 by the international agency for research on cancer (IARC, 1987) found that there is sufficient evidence in humans for carcinogenicity of naturally occurring mixtures of aflatoxins. The aflatoxins are therefore classified as Group I carcinogens. Acute aflatoxins in humans has been reported in Kenya (Ngindu et al., 1992; CDC, 2004) Uganda (Alpert et al., 1971), Mozambique (Van Rensburg et al., 1974), Swaziland (Peers et al., 1976), Transkev (Van Rensburg et al., 1990), Makaula et al.,1996; Jaskiewictz et al.,1987). A recent review showed that the danger of aflatoxicoses cases still exists in Africa (Odamtten, 2005).

However, rather curiously, analysis for aflatoxins showed that aflatoxins B_1, B_2 G_1, and G_2 were not formed (Table 5). Presumably, the raw spices and spice products were stable and their formulation and preservatives,

offered self-protection against aflatoxins formation by *A. flavus* especially, in royco and maggi cubes which might have produced conditions unfavourable for aflatoxins formation. It may also be possible that the strain of *A. flavus* used does not produce the toxins under certain conditions. Further studies on this strain are required to ascertain its mycotoxigenic potential.

To ensure effective food safety management in Africa, tolerance levels of mycotoxins and bacteria should include measures to control mycotoxin formation through good agricultural practices, proper storage and handling methods (Odamtten, 2005). Food safety control measures should also be used at each stage in the production chain as prescribed by the hazard analysis critical control point (HACCP) concept. Other strategies such as the use of appropriate processing techniques, simple cleaning and segregation procedures could help reduce levels of contamination of foods for human consumption. The highest priority however, needs to be placed on creating consumer awareness and adequate education for producers of agricultural produce (IAEA, 2004).

Even after using HACCP to select samples for storage, samples may be predisposed to varying environmental humidities that are unsafe. It is therefore expedient to eliminate microorganisms using methods other than chemical applications. Chemical applications are now banned in many countries. Gamma irradiation of food has been proposed and is being used in 26 countries worldwide to curtail the resident microorganisms in foods and thus extend the shelf- life of many agricultural products (IAEA, 2004). The recommendation by the joint FAO/ IAEA/WHO experts committee of food Irradiation accepted in 1980, as safe for human consumption, products treated with an overall gamma irradiation dose of 10 KGy. Irradiation of spices improved stability of products and goods produced from them (IAEA, 2004).

Conclusion

It has been suggested that a combination of moist heat (applied at ≤ 60°C for 5 min at ERH of 85%) prior to irradiation has a higher synergistic killing effect on spores of microbial origin (Odamtten et al., 1985c, 1986b, 1987; Brodrick et al., 1977; Langerak and Canêt–Prades, 1979) and can curtail *A. flavus* growth and toxin production. Future studies will look at the combination treatment in order to advance this present study and make it widely applicable in the food industry.

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